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Rice Hull Bioreactor for Recirculating Aquaculture

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RICE HULL BIOREACTOR FOR RECIRCULATING AQUACULTURE

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ABSTRACT

The engineering of floating media biofilters has been optimized over the years. The backwashing process has made them more energy and water efficient. Likewise, moving bed bioreactors (MBBR) are gaining interest and popularity because they are relatively affordable to build. Yet, developing countries' aquaculture production remains largely excluded from the advances made in recirculating aquaculture systems (RAS). This discrepancy is partially driven by the high costs of media such plastic beads and Kaldnes (KMT) media, commonly used in MBBR.

This dissertation evaluates the usability and profitability of rice hulls (RH), an abundant by-product in many developing nations, as a sinking biocarrier in a 3-phase filter system for low loading applications. The hulls have a shape, structure, and specific surface area of 1850 m^2/m^3, comparable to synthetic plastic "EN" beads used in PolyGeyser® tank filters. Yet, their production cost is negligible.

A lab experiment compared the TAN removal capacities of RH and EN media in a 3-phase reactor configuration, in ultra-oligotrophic, oligotrophic, and lower mesotrophic conditions. The RH displayed a volumetric TAN conversion rate (VTR) of 1025 g-N/m^3, and the EN displayed a VTR of 1219 g-N/m^3, proving RH to be a viable biocarrier. RH partially degraded after 18 days in the reactor, and were evacuated as sludge. Because of their small size and sinking properties, RH reactors require an internal clarifier to keep media inside the reactor.

A commercial-scale recirculating aquaculture system (RAS) with a RH bioreactor (RHBR) was designed for tilapia fingerling production, based on a conservative RH VTR of 700g_N/m^3. A cost analysis showed that ownership costs for a facility using this RAS were \$0.11/lb. for fingerlings in the U.S. or Europe, and \$0.06/lb. for a fingerling facility in a

developing country with abundant availability of fish and rice. Operation, maintenance, and storage associated with media replacement are the major drivers of costs. A comparative analysis showed that while an RHBR is not significantly profitable in most western countries, it shows promising potential in rice-producing developing countries, allowing them to opt for a more affordable integration of modern biofiltration in their aquaculture industries.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Overview

In 2011, Davis *et al.* conducted a series of experiments to determine rice hulls' (RH) potential for nitrogen oxidation in wastewater treatment. They used the husks as biocarrier media in a hanging basket, trickling filter system. Synthetic, clarified, wastewater with high initial ammonia concentrations was sampled for 10 days. Results showed development of a mature biofilm, and promising oxidation rates. Nevertheless, the authors called for further, longer research not only for wastewater treatment, but also for aquaculture, as presented in the present dissertation. This project is also in dialogue with Gutierrez-Wing and Malone (2006) who predicted ten years ago that the "demand for cost-effective biofilters will increase with the expansion of [recirculating aquaculture systems (RAS)]." White *et al.* (2004) further reported that with the blue revolution, mass-scale aquaculture too often relied on systems that are "environmentally and socially damaging." This project is in agreement with the latter's call for "overdue reform" in aquaculture production to utilize technology that is sustainable, modern, and efficient, yet economically feasible worldwide and non-socially invasive.

Moving bed biological reactors (MBBR's) are innovative approaches. They are proven systems that have demonstrated satisfying nitrogen removal rates at high loadings, and for intensive applications. They comprise a submerged biofiltration system which media bed is continually expanded via hydraulic, mechanic, or pneumatic motion. Whether in aquaculture water or in more polluted wastewater, the typical media used in the Kaldnes (KMT) cylinder or media of similar shape and configuration. While several studies have compared Kaldnes/MBBR with different systems equipped with other media, the potential of a moving bed configuration with another media is yet to be explored. Furthermore, KMT-mediated MBBR's are proven and

well established to remove nitrogen at high loading concentrations, but not in lower trophic (ultra-oligotrophic, oligotrophic, mesotrophic) applications such as hatcheries, nurseries, and fingerling and ornamental aquaculture.

On the other hand, floating bead filters (FBF's) utilize plastic, low-density media. The media is typically spherical, and varies in diameter. Recently, Aquaculture Systems Technologies developed an enhanced bead. This oval shaped media is intricately carved for crossflow and increased surface area. This design allows for greater nitrification, hence the name Enhanced Nitrification (EN) media. Comparative studies (Malone *et al.*, 1993; Wagener, 2003) concluded in EN/FBF superiority in nitrogen removal. While EN beads are a proven media in FBF, their ability to nitrify in other biofilter systems has been seldom assessed (Bellelo, 2006), particularly in aquaculture.

1.2. Goals and hypotheses

To help fill this gap in data, this dissertation sought to evaluate the nitrification capacity of a moving bed reactor that uses alternative biocarriers in ultra-oligotrophic through mesotrophic aquaculture water, and to determine the economic feasibility of its implementation in developing countries. This research addresses the need for environment-friendly media primarily by designing a filter that utilizes organic materials as biocarrier: rice hulls (RH). A laboratory experiment was designed to determine sizing criteria for this media. For validation purposes, a comparison of RH performance with that of another acknowledged media, Enhanced Nitrification (EN) beads, which are modified polyethylene beads commonly used in floating bead filters (FBF's). In order to draw focus on media behavior rather than system differences, the utilization of EN in a moving bed filter design as similar as possible to the filter designed for

RH. The goal was to determine which biocarrier had the greater TAN removal capacity, and whether an RH design was worth pursuing.

As RH proved to be a valid biocarrier, the need is addressed for affordable aquaculture in developing countries, where rice husks are a material available locally and abundantly, by investigating the economic feasibility of commercial application of an RH-mediated RAS. In this framework, the first goal was to develop modified moving bed filter designs with EN and RH biocarriers, fit for commercial-scale recirculating aquaculture applications. Comparative cost analyses to were conducted to compare a) the costs of EN-mediated RAS facility with the costs of a RH-mediated RAS in a western country like the US; b) the costs and affordability of EN v. RH-mediated RAS facilities in a developing country with growing aquaculture production; c) the costs and affordability of EN v. RH-mediated facilities in a developing country with under-developed aquaculture production.

These deliverables aim at fostering discussion and opening a dialogue on the affordability of RAS, self-reliance in the developing world, engineering parameters for green aquaculture technology, and the overall adaptability and versatility of different media and biofilter configurations.

1.3. Organization of the dissertation

Chapter 2 provides insight regarding the existing literature as well as past and recent research that has contributed to the knowledge produced in this dissertation. Chapter 3 offers biofilter designs for expanded biofilters with RH and EN carriers. This section also reports results from laboratory experiments that tested the filtering capacity of RH as a carrier, in comparison with EN beads for low-loading applications. Subsequent calculations determined the feasibility of RH-equipped reactors. Chapter 4 is a follow-up study that uses data from Chapter 3

to design RH and EN-mediated reactors modified for commercial scale aquaculture. Data from Chapter 3 and prior studies helped establish a cost-analysis of three hypothetical RAS-equipped facilities that operate with RH and EN as carriers. Costs were successively adjusted based on the facility's location: the US, India, and Côte d'Ivoire. Finally, Chapter 5 synthesizes all results and provides suggestions for future research, as well as recommendations for aquaculture and wastewater treatment applications based on the information provided in this dissertation.

CHAPTER 2: BACKGROUND

2.1. The concept of biofiltration: aerobic v. anaerobic processes

Biofiltration is the process by which dissolved organic waste is removed from the nutrient-rich wastewater via bacterial activity. The environment in which biofiltration occurs (whether in nature or in an artificial space), along with its components, is commonly referred to as the biofilter. Segments of this process may take place aerobically (in the presence of oxygen) or anaerobically (absence of oxygen).

Heterotrophic bacteria are responsible for organic oxidation. They get their energy from the organic compounds found in the water. They also consume dissolved oxygen from the air and other sources to which they are exposed (O_2). Bacteria spread and grow onto a media (natural or artificial) material present in the water to form a biofilm where particle diffusion can occur. Anaerobic biofiltration occurs in watertight filters, such as septic tanks, that function as digestors. Depending on their diameter/length ratios, biodigestion in anaerobic reactors can enhance the treatment of effluents with high organic loads (Kunzler *et al.*, 2013), even at low and high temperatures (Oleszkiewicz, 1981; Oleszkiewicz and Koziarski, 1982). Nevertheless, the study of Sauvegrain *et al.* (1992) suggested that while both aerobic and anaerobic membrane filtration exhibit comparable BOD removal efficiency, aerobic filtration has higher COD and TAN removal efficiency. Rebah *et al.* (2010) confirmed aerobic biofiltration's capacity to remove carbonaceous and nitrogenous pollution and phosphorus at high rates and with low energy. Anaerobic filters are thus recommended for waters with narrow COD to BOD ratios and few suspended solids. On the other hand, aerobic biofilters can purify water with high densities of organic wastes from fish excretions for example (Colt and Armstrong, 1981; Rebah *et al.*,

2009). They are particularly appropriate for aquaculture water filtration with high loadings (TAN).

In fixed-film recirculating aquaculture systems (RAS), aerobic biofiltration includes an organic oxidation process, during which organic waste material from the marine species' metabolic activity is exposed to air (oxygen). Oxidation not only gives the bacteria enough oxygen to survive, grow, and spread onto the media, but it is also the process by which the organic material (the ammonia-rich excretions from the marine species cultivated) exhibits its biodegradability. Thus, as the oxidation equation shows, the amount of organic carbon produced shows how much O_2 is needed and subsequently what would be an appropriate biofilter (hence facility) size:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

Nevertheless, biodegradation alone is typically not rapid enough to eliminate waste. Actually, the rate at which aerobic biodegradation occurs is often not enough to counter the rate at which protein decay within the organic waste occurs.

During biofiltration, oxygen is needed for bacteria cell's respiration and growth. Proper oxygen supply helps maintain biochemical energy production. Oxygen is transported via air bubbles. RAS filtration systems typically include air stones (usually made of ceramic, alumina, or Teflon, with a porous surface), tubing, and other aeration devices for oxygen transport. Its transfer from gas phase to the growth medium can be described mathematically (Piret and Cooney, 1991; Roy *et al.*, Garcia-Ochoa and Gomez, 2009) in terms of transfer rate.

2.2. Suspended growth and fixed film systems

Biofilters function as a bioreactor where bacteria that break down the organic materials in the sludge are activated to proceed to a series of chemical reactions. Biofiltration can occur in

suspended growth systems, where the activity of bacteria occurs in flocks of suspended microorganisms[1]. Bacterial and microorganic activity can also occur as a fixed film that grows onto a solid present in the wastewater. The item onto which the film attaches is called the media. Suspended growth systems are usually more sensitive and unstable than fixed film systems, due to constant water motion.

While biofilters in RAS are designed to treat water internally, suspended growth systems are designed for the microorganisms to remain in suspension (Liang *et al.*, 2014; Fernandes *et al.*, 2016; Luo *et al.*, 2017). This form of aerobic biofiltration is characterized by constant bacterial growth within nutrient-rich waters. Yet, the suspension layout is often unstable and promotes poor water quality. It therefore requires heavy management. Despite higher initial costs than suspended growth systems, fixed films are a stable thus time-efficient system favored in the aquaculture community as it is subject to perform at low operation costs (Gutierrez-Wing and Malone, 2006).

Hence, several agricultural and aquaculture applications have elected a combination of aerobic and anaerobic processes for wastewater and tank water filtration as an alternative to suspended-growth systems (Oleszkiewicz, 1981; Chiou *et al.*, 2001; Rebah *et al.*, 2009).

2.3. Heterotrophic bacteria and nitrifiers

The aforementioned oxidation equation ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$) details how heterotrophic bacteria metabolize organic matter ($C_6H_{12}O_6$) with oxygen consumption through a process that produces water (H_2O) and releases gaseous carbon dioxide (CO_2). In aerobic biofiltration, nitrogen processing is characterized by an increase in total ammonia nitrogen

[1] Despite the presence of several microorganisms, biofiltration processes are mainly related to bacterial activity.

(TAN) that results from toxic protein decay. Thus, in addition to being biodegraded by air during oxidation, the presence of bacteria and other microorganisms help speed up the purification process. Bacteria ingest the organic waste. The by-product of bacterial digestion is nitrate (NO_3), a less toxic form of nitrogen. This oxidation process is referred to as nitrification, as the bacteria oxidize the ammonia.

The biofilm is a thin layer of bacteria. Slimy in texture, and adheres to a biocarrier's surface. Bacterial biofilms are constituted in layers with the deeper layers closest to the media. They become increasingly anaerobic as the film gets thicker, but the entire biofilm gets more anaerobic as it reaches closer to the media. The outer layers are the aerobic layers where the bacteria grow the most. In the middle are the meso layers. These receive less oxygen that the aerobic layers, which is conducive to endogenous response. Despite the layer pattern, biofilm properties should not be assumed to be of uniform distribution (Zhang *et al.*, 1994; Zhang and Bishop, 1994). Zhang *et al.* (1994) measured the dissolved oxygen penetration to be about 600 µm for a film with a thickness of 1319 µm. Nevertheless, DO concentration decreased as the organic loading rate increases, until this rate exceeded a certain value after which the biofilm's oxygen profiles remained the same. Zhang and Bishop (1994) microsliced biofilms into layers 10 to 20 µm thick to determine the effect of biofilm composition and structure on bacterial activity. The bottom layers were measured to be 4 to 7 times denser than the top layers. Thicker biofilms have a large discrepancy in the number of active bacteria at the top (82-89%) and at the bottom (5-11%). The films' porosity also decreases considerably from the top to the bottom layers in thick and thin biofilms, leading the authors to state that "the ratio of effective diffusivity to diffusivity […] shows a decrease with depth of the biofilm."

Biofilters are hence the site where the nitrifying bacteria convert ammonia dissolved into nitrites. Consequently, in terms of design, the biofilter's sizing must be engineered based on the unit's nitrification capacity. For a filter such as the PolyGeyser®, the filter chamber (that contains the media) and its charge chamber are fluidly connected as the latter accumulates air in order to agitate the floating media (Malone, 2003).

In aquaculture, biofilms help sustain good water quality thanks to the "uptake of nitrogen compounds and the production of high levels of dissolved oxygen associated to the proliferation of autotrophic microorganisms" (Viau *et al.*, 2015). The bacteria population in the biofilm is made up of autotrophic and heterotrophic organisms. The autotrophs that produce their own energy grow first (Viau *et al.*, 2015) and are the primary nitrifiers (Zhang *et al.*, 1994). They must compete with the heterotrophs for substrate space and for oxygen in the biofilm. This competition leads to the films' layered structure and non-uniform distribution within the biofilm. Zhang *et al.* (1994) observed that competition is heightened when dissolved oxygen levels in tank water are low (due to high loading). Furthermore, the authors noted that when nitrifiers suffer oxygen shortage – either because of high loading or because of competition with heterotrophs – nitrification is inhibited.

2.4. Clarification and biofiltration in recirculating aquaculture systems (RAS)

The five major processes of recirculating aquaculture are, as shown in Figure 1: clarification, biofiltration, circulation, aeration, and degassing. Vilbergsson *et al.* (2016) compiled a detailed taxonomy specifying means and ends that address each of these processes. RAS are designed with clarifiers and biofilters that specifically address the fouling of tank water from defecation, sedimentation, and other solids capture (Malone, 1993; Malone, 1995; Malone, 2003; Greensword, 2015).

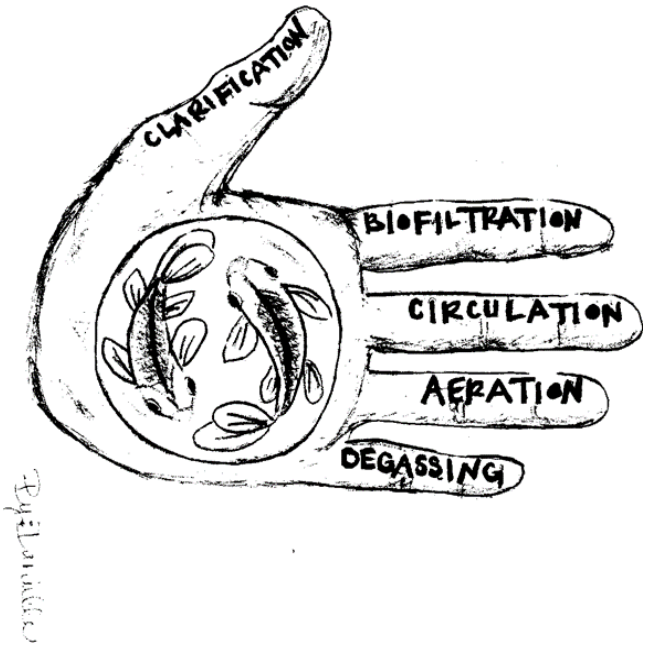

Figure 1: The five fundamental processes of RAS, as presented by Malone and Gudipati (2005)

A clarifier is the location where the collected suspended sediments settle and thicken before being removed via the sludge chamber. The clarifier's most important parameters are retention and surface area (which determines the hydraulic loading). Retention time is dependent upon the water throughput. Retention time numerical value indicates how long the wastewater will stay in the clarifier, that is, the time needed for settling. The hydraulic loading value provides the correlation between the clarifier's surface area and flow rate. A typical aquatic hydraulic loading rate is 0.25 pound per ft^3 (Malone and Beecher 2000). Clarifiers feature some means of removing solids, which should generally be performed before biofilm forms. This limits the growth of heterotrophic bacteria and organic carbon accumulation. It also facilitates the growth of ammonia-oxidizing bacteria, as well as nitrite-oxidizing bacteria (Ebeling, 2006).

Biofiltration is the subsequent breakdown of organic and inorganic materials, aerobically or anaerobically, that had been previously removed through clarification. Solids management and capture in aquaculture waters is completed via fluidized beds, bead filters, microscreens, and other membrane biological reactors (Burden, 1988; Thomasson, 1991; Sandu *et al.*, 2002; Malone and Gudipati, 2006; Malone and Beecher, 2000; Ebeling *et al.*, 2003; Ebeling *et al.*, 2004; Summerfelt and Penne, 2005; Sharrer *et al.*, 2006; Sharrer *et al.*, 2010). Fluidized beds are made up of small particles (sand or plastic beads) that adopt fluid-like properties. PolyGeyser® filters are filled with a plastic floating device and a layer of moving plastic beads. Biological water treatment requires the use of bacteria that assist in the removal of toxic nitrogen.

The nitrogen cycle takes place in three stages: nitrogen fixation, nitrification, and denitrification. During the first stage, as proteins decay, symbiotic nitrogen-fixing bacteria, including cyanobacteria, convert nitrogen into inorganic ammonia, using hydrogen:

$$N_2 + 3H_2 \rightarrow 2NH_3$$

2.5. Nitrification in RAS

Nitrification, the second stage of the nitrogen cycle is of higher interest in biofiltration because as ammonia nitrogen primarily characterizes waste that is to be removed. Nitrifying bacteria get their energy from oxidized inorganic compounds of nitrogen. Types include ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). Organic oxidation helps facilitate nitrification because it releases carbon dioxide consumed by these autotrophic bacteria. *Nitrosomonas* and *nitrobacter*, among others, oxidize the toxic ammonia into nitrites. The bacteria then convert nitrites into nitrates. The nitrate-rich water is less toxic, but the tank still needs to be cleaned and have its water replaced.

Ammonia oxidizing autotrophic bacteria such as *nitrosomonas*, *nitrosococcus*, and *nitrosolobus*, get energy from inorganic compounds. They use bicarbonate present in the water and air to oxidize ammonia. They catabolize un-ionized ammonia (NH_3). As ammonia ionized into NH_4^-, the bacteria form nitrite (NO_2):

$$NH_4^+ + 2\ H_2O \rightarrow NO_2^- + 8H^+ + 6e^-$$

and

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O$$

NOB such as *nitrobacter*, *nitrospira*, and *nitrospina* then oxidize nitrite to form nitrate (NO_3)[2]:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

Nitrogen is transported by a diverse group of microorganisms in aquacultural water. Researchers have studied and characterized a large number of these nitrifier communities (Cai *et al.*, 2012; Brown *et al.*, 2013; Kumar *et al.*, 2013). Cai *et al.* (2012) identified (via phylogenetic analysis) a cluster of 19 Operational Taxonomic Units (OTUs) from the Proteobacteria and Planctomycetes bacterial library in aquacultural systems. Proteobacteria that made up 99.2% in an artificial marine ecosystem. Biodiversity is essential to ensure effective nitrification (van Kessel *et al.*, 2010).

Blancheton *et al.* (2013) account for the bacterial diversity in RAS based on biofilm type. Tal *et al.* (2003) determined that marine and freshwater RAS also influence the nitrifying bacterial composition. Additionally, in an RAS, the ammonia-oxidizing populations often differ based on the species in the tank. For example, Brown *et al.* (2013) characterized the nitrifier community in a shrimp RAS biofilter. In the light of these findings, nitrifying bacterial products

[2] Ebeling (2006) provides the amount of energy in kcal/mole of ammonia or nitrite produced in each of these stages of nitrification.

are commonly manufactured and purchased to help improve an RAS' nitrification efficiency. Kumar *et al.* (2013) characterized the molecular composition of the bacterial consortia used for different RAS' bioreactor activation. Nitrifying autotrophic bacteria coexist with heterotrophic, but the two groups compete for space and oxygen. For instance, if there are too many suspended solids (organic material), heterotrophic bacteria will use too much space and oxygen for autotrophic bacteria to survive and complete nitrification. It is thus recommended that total suspended solids (TSS) concentrations be low in the bioreactor.

Nitrification needs are commensurate with the trophic level demands of the species cultivated and the scale of production. The nitrite-N concentration below 1 mg/L (TAN) determines the nitrification capacity of a biofilter. (Zhu and Chen, 2001). Yet, while oligotrophic systems have a maximum TAN of 0.3 mg-N/L, marine larval system cannot tolerate TAN above 0.1 mg/L. These freshwater systems such as fingerling industries require biofiltration at what is called an ultraoligotrophic level. The biofilter sizing criteria for such systems must be drastically increased (Gutierrez-Wing and Malone, 2006; Malone and Beecher, 2000).

2.6. Denitrification

Nitrates present after nitrification are still toxic (Liao and Mayo, 1974; Otte and Rosenthal, 1979; Bovendeur *et al.*, 1987). Denitrification completes the nitrogen cycle; it is the process by which a variety of anaerobic bacteria, fungal species, and other microorganisms complete nitrate breakdown, which allows nitrogen to be released back into the atmosphere in gaseous form.

Denitrification may also require media for bacterial and microorganic growth, and it can be completed artificially by adding chemicals that neutralize the nitrates. Saliling *et al.* (2007)

considered wood chips and wheat straw as denitrification media for alternative biofilter device in aquaculture and wastewater treatment.

2.7. Factors affecting nitrification

Biofilter sizing in terms of TAN must take into account the fact that not all TAN from protein decay translates into nitrate in the nitrogen cycle. A portion of it converts into biomass, which is necessary since bacteria feed on nitrogen in order to grow and spread. Several other factors influence nitrification and the nitrifying bacterial activity: water temperature, hardness (alkalinity), pH, D.O., and the concentration of chemical components in the tank water.

Emparanza (2009) stresses the importance of biofilter management, especially in commercial-size RAS. Morales *et al.* (2015) studied the impact of carbon source on bacterial activity. They found that organic carbon's removal efficiency (50%) was higher than that of inorganic carbon (45%). Furthermore, the study of Scott (2002) compared the nitrification rates of 3 different types of plastic media based on organic loading. This shows that media type is also an influential factor on bacterial activity.

For fixed film biofilters, optimal pH conditions range between 7.2 to 8.8 for AOB and 7.2 to 9.0 for NOB (Chen *et al.*, 2006). Low pH inhibits nitrification due to hydrogen ion toxicity (Szwerinski *et al.*, 1986), although pH decreases naturally as you get deeper into the biofilm.

Moreover, alkalinity refers to the extent to which the water is capable of neutralizing acids and buffering changes in pH. Nitrification usually results in alkalinity decrease as AOB oxidize ammonium ions. While the effect of nitrification on alkalinity is well-documented, few studies have distinctly explored the inverse influence of alkalinity on nitrification. Bai *et al.* (2010) suggested that in high alkalinity conditions, aerobic nitrification was faster in closed systems than in open systems. Biesterfeld *et al.* (2003) evaluated different alkalinity types

(carbonate, phosphate, carbonate and phosphate, and phosphate and hydroxide) and concluded that carbonate alkalinity is necessary to ensure AOB's access to inorganic carbon in order to grow and complete cellular synthesis. Such alkalinity is needed in addition to alkalinity previously administered as acid neutralizer. The study of Peng *et al.* (2003) paralleled alkalinity and pH's effect on nitrification rates. They suggested that while pH can be used as a control tool to manipulate nitrification time, the focus should be shifted from pH as a control to alkalinity instead.

Regarding temperature conditions, Zhu and Chen (2002) exposed the importance of generating equations specifically for fixed film biofilters. While higher temperatures provide enhanced nitrification rates in suspended growth systems, few studies have documented or determined a mathematical model for the impact of temperature on moving bed nitrification. Nevertheless, the study of Zhu and Chen (2002) detailed that high temperatures limit oxygen availability. Subsequently, lower DO concentrations at saturation showed that higher temperatures impact nitrification negatively in fixed film systems. Furthermore, Saidu (2009) who specifically addressed temperature impact on a bead media-equipped system, reported that ammonia utilization rates increased at higher temperatures, but the TAN removal rate did not.

Chen *et al.* (2006) gathered the literature available to generate a table that summed up the different impacts of DO on nitrification. They concluded that, because fixed films have unique transport of dissolved nutrients and oxygen (diffusion), DO concentrations must remain high in reactors.

Water turbulence can also hinder nitrification. Indeed, in waters with high velocity, the biofilm into which the mass transfer of nutrients and substrate occurs, cannot thicken to optimal

thickness (de Beer *et al.*, 1996). Depending on their types, some filters require that sloughing occur at a given peak hydraulic rate, and water turbulence.

High salt concentrations tend to slow down the AOB nitrification stage during media acclimation in marine systems (Gutierrez-Wing and Malone, 2006; Manthe and Malone, 1987; Svobodova *et al.*, 2005). Salinity as a factor is often considered negligible or assumed constant for simplicity (Malone and Pfeiffer, 2006). Yet, several studies have investigated salinity as a parameter of acclimation speed (Nijhof and Bovendeur, 1990), bacterial production (Pakulski *et al.*, 1995), and nitrogen removal (Fontenot *et al.*, 2007) in RAS. Nijhof and Bovendeur (1990) measured that marine systems have a considerably slower nitrification capacity, with an ammonia removal rate 40% that of freshwater RAS. Fontenot *et al.* (2007) determined that salinity 28-40 ppt generated best nitrogen removal in an activated sludge biological treatment of shrimp. Pakulski *et al.* (1995) measured bacterial production at different salinities and determined that intermediate salinities (10-27‰) were optimal for nitrification in freshwater systems. The study of Kuhn *et al.* (2010b) showed that natural sea salt can be substituted with synthetic salts in a RAS with moving bed biofilters.

Fixed film bioreactors are characterized by the thin bacterial biomass that lines the media and the dissolved nutrients; and the transportation of dissolved nutrients and oxygen into the biofilm via diffusion. For submerged fixed film biofilters, the filter allows the circulation of oxygen-rich water when it reaches the biofilm. This is why the recirculation rate or air input into the water must be high. Nevertheless, a fixed film biofilter's capacity is expressed in terms of ammonia diffusion, not in terms of D.O. Fixed film biofilters differ in their strategy in which they provide oxygen and limit the growth of excess biofilm (Malone and Pfeiffer, 2006). Media decay rate should also be taken into consideration, and the media should perform some level of

abrasion in order to keep flocculation from accumulating too widely. Other required characteristics include, among others, suspension.

To assess a bioreactor's efficiency, one should look at elements that affect nitrification rates. Scott (2002) demonstrated that organic loading is a reliable source of data.

2.8. Biofilter classification

Fluidized beds, moving bed biological reactors (MBBR's), and floating bead filters are common aquaculture filtration devices (Burden, 1988; Thomasson, 1991; Sandu *et al.*, 2002; Brindle and Stephenson, 1996; Malone and Gudipati, 2005; Malone and Beecher, 2000; Sharrer *et al.*, 2007; Sharrer *et al.*, 2010). Malone and Pfeiffer (2006) detailed a biofilter classification of floating bead filters. Moving media RAS filters like the moving bed reactor (Ødegaard *et al.*, 1994; Rusten *et al.*, 2006) or the microbead filter (Timmons and Summerfelt, 1998) contain a mix of water and air that move the filtering media through constant motion. RAS filters with static beads, do not operate in media motion. Instead, the water goes through a stationary media bed. Propeller- wash filters (Malone and Beecher, 2000; Chitta, 1993), hydraulic filters (Wimberly, 1990), and the bubble-washed (Sastry *et al.*, 1999) are examples of such stationary media systems. They differ by their washing technique. Saidu (2009) also demonstrated that bead biofilters tend to perform more efficiently as temperatures increase. A rice hull-equipped RAS would be a 3-phase, fixed film reactor, following the classification detailed in Figure 2.

Suspended-growth bioreactors (SGB's) promote the suspension of microorganisms as the reactor is mixed (pneumatically aerated or mechanically agitated). Microorganisms attach to one another with polysaccharides and protein bonds, forming activated sludge. Two major configurations of suspended-growth bioreactors are being considered by the aquaculture industry: sequencing batch reactors (SBRs) and membrane batch reactors. Kuhn *et al.* (2010a)

detailed the different stages of SBR operation. During the fill stage, the loading-rich wastewater is “pumped or gravity fed into the SBR.” At this point, the activated sludge from the previous batch cycle is already inside the bioreactor. During the reaction stage, the “dirty” wastewater mixes with the sludge until the desired amount of nutrient removed is achieved. The settling stage occurs after this mixing as the sludge settles out of the “quiescent water column.” The decantation stage consists in removing the treated, “clean” wastewater from the reactor as the settled sludge remains inside the reactor. Despite the high performance of SGB’s, their efficiency can be limited in instances of excessive suspended solids or exceedingly high nitrate levels.

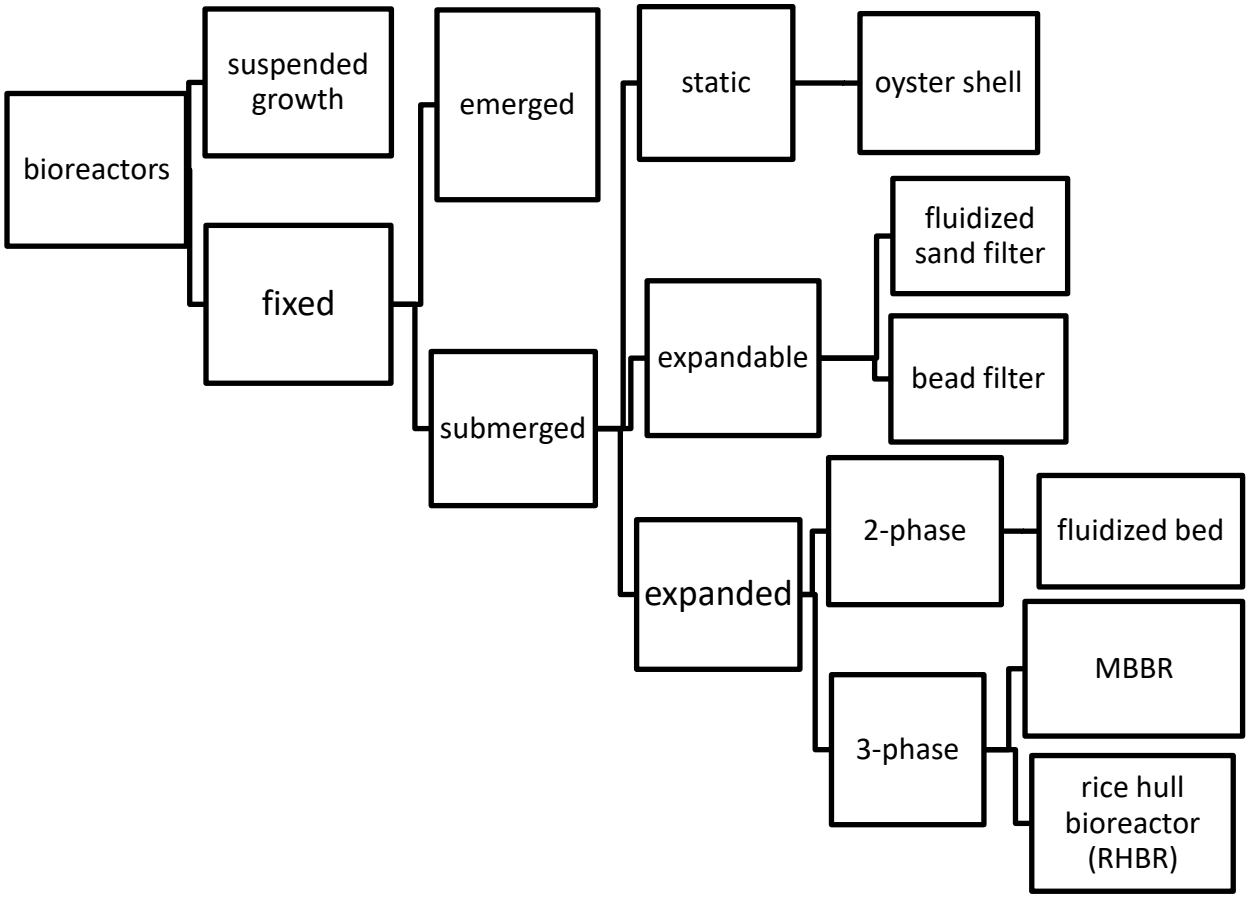

Figure 2: Classification of submerged bioreactors

With fixed growth systems, cell cultures' spread is limited because "static cultures deliver suboptimal nutrition/waste exchange conditions" (Caicedo-Carvajal *et al.*, 2012). The media do not move and the media bed remains stationary as the water goes through it. Other systems have an expandable surface for enhanced culture cells' growth. Fluidized-sand filters are ideal to remove dissolved wastes in cool and cold water conditions (Summerfelt *et al.*, 2003, 2004a, 2004b). Bullock *et al.*'s (1993) report indicated that the shape of the media and the scouring effect of the sand particles affected not only oxygen levels during filtration, but also the type of bacteria that can grow on the media. Summerfelt (2006), Timmons *et al.* (2000), and Malone and Pfeiffer (2006) proposed several alternative, innovative sand biofilter designs and supportive systems.

Expanded systems can be 2-phase reactors that reflect a process with two phases: liquid and solid (organic and biosolids). Fluidized beds are preferred devices for anaerobic treatment. The water is passed through some granular material (beads, sand) at high velocity which causes the solid to suspend and move with fluid-like properties. A distinctive feature of fluidized bed reactors is their fixed film process that uses hydraulically suspended sand (or plastic) as a biocarrier (Summerfelt, 2006; Weaver, 2006). Fluidized bed filtration removes pollutants on large surface areas, ideal for high quality, oligotrophic water conditions (usually required for spawning and larval rearing). Weaver (2006) noted that fluidized beds are particularly effective with soluble components removal. He also reported that fluidized bed nitrification optimized when combined with a floating bead filter. 2-phase reactors do not utilize air, which would otherwise help the bacteria grow and spread. The 2-phase RAS are relatively affordable.

MBBR's are 3-phase reactors with moving media which motion is also promoted by the mixture of air and water at a constant pace. The 3 phases refer to air, water, and biosolids.

MBBR's are relatively affordable to build. They have an expanded, submerged biofilter (Malone and Pfeiffer, 2006). Typically designed for fixed film media (Malone, 2013), the distinction between MBBR's is based on the biocarriers they utilize. Although MBBR's are multimedia system, most of them use KMT as biocarrier (other carriers include foam balls and other media). The media can be buoyant, if it is submerged yet prone to floating (such as foam balls that have low density). Moving bed reaction is also characterized in terms of whether the fixed film reaction is internal or external, that is, whether the bacteria grow inside or outside the media. 3-phase reactors reflect a typically aerobic process: gas, liquid, and solid. Oxidizing bacteria constitute the biocatalyst contained in the solid phase. Oxygen (air) and released carbon dioxide constitute the gas phase. Air helps improve circulation of bacteria (and increase bacteria growth), as well as liquid circulation. Inversely, the liquid helps air circulation through diffusion to help maximize aeration. In RAS, the air is typically generated by the pump, which results in higher costs than 2-phase reactors would require. Liquid holdup is a common hindrance in expanded 3-phase reactors. Yu and Rittman (1997) provided an algorithmic model to help predict and prevent such holdups. 3-phase expansion depends on the gas and liquid injection rate (velocity) and the catalyst's size and shape (Soung, 1978).

2.9. Biocarriers

Since the media is the biocarrier onto which the microorganisms attach, the media's surface area should be optimized for maximum nitrification. Effective media help reduce fouling in the moving bed's biofilm reactor while these purifying biocarriers can have different shapes, surface areas, and textures. Ebeling (2006) explained that cross-flow media have a better ammonia removal rate than vertical or random flow media that tend to clog more easily. The study of Al-Hafedh *et al.* (2003) concurs by establishing the superiority of pipe-shaped media

over other non-pierced media. Media materials include sand, rocks, and shells, but Al-Hafedh *et al.* (2003) suggest that plastics constitute one of the best media materials both in terms of TAN removal and in terms of cost.

Viau *et al.* (2016) tested several uncommonly used media substrates with shrimp tanks, namely polyethylene nets, agrovelo (plastic films used to protect plants from frost), and plastic bottles, to explore innovative sustainability and recycling options for domestic and agricultural waste products. All substrates generated similar nutritional values in terms of lipids and protein content. Agrovelo generated the best water quality, but plastic bottles emerged as the most beneficial substrate because of its reusability. In addition, it has no production cost and it is readily available from common municipal solid waste. There is an effort in the aquaculture community to develop innovative media that are cost-effective and environment-friendly.

2.9.1. Enhanced nitrification (EN) media

EN media are typically used in submerged, expandable floating bead filters (FBF's). This biocarrier has been widely studied and its efficiency is scientifically acknowledged. Mostly designed as a cross-flow floating media, EN display considerably low headlosses at flow rates used for high rate nitrification. Nevertheless, reports concur in their statements that it is an expensive media (Fadhil *et al.*, 2011; Timmons *et al.*, 2006; Ebeling, 2006). Bellelo (2006) studied filter configuration of Static Low Density Media (SLDM) in high-density RAS-like domestic wastewater treatment with EN and Kaldnes Miljøteknologi (KMT) media (Figure 3) used as a packed bed. In post-primary clarification BOD_5 measurements, (carbonaceous biochemical oxygen demand) and TSS (total suspended solids) concentrations, EN reduced 90% both BOD_5 and TSS. KMT reduced both 10% less than EN. EN's superior TSS removal can be associated with FBF's ability to simultaneously capture solids and provide biofiltration (Malone

et al., 1993). Yet, in Bellelo's (2006) study, KMT generated less than half of EN's oxygen uptake, because of its lesser specific surface area (SSA) per unit volume (EN beads have a SSA of 1100-1450 m^2/m^3). EN media also displayed low headloss even at high flow rates. Despite media differences, filters had identical or similar configurations. Using a non-traditional configuration (EN are usually associated with expandable FBF's, and KMT is usually associated with expanded MBBR's) helped demonstrate the relevance of the media itself. Guerdat *et al.* (2010) also conducted a comparative analysis that confirmed the superiority of EN beads in a PolyGeyser® over a KMT system in terms of VTR, as they exhibit a VTR of 750 g-N/m^3 (Malone and Pfeiffer, 2006). Although the PolyGeyser® exhibited a lower VTR than the considered fluidized sand filter, Guerdat *et al.*'s (2010) statistical and prediction analyses showed that the bead filter mathematically displayed better removal rates at high TAN loading rates.

2.9.2. Kaldnes (KMT) media

The most commonly used MBBR media is the 3-D polyethylene disk commercialized by the company AnoxKaldnes. The wheel-like media is spoked, allowing for cross-flow and both internal and external bacteria growth (Rogers *et al.*, 2010). This cylindrical plastic media was originally supplying wastewater treatment systems (including trickling filters), for which it remains a favored media for its ability to remove nitrogen with high loadings (Ødegaard *et al.*, 1993; Ødegaard *et al.*, 1994; Ødegaard *et al.*, 2000; Metcalf and Eddy, 2003; Wagener, 2003; Lekang and Kleppe, 2000; Rusten *et al.*, 2006). The research of Rusten *et al.* (1994) indicated that KMT performed best at high loading concentrations, as VTR ranged between 300 and 400 g-N/m^3/day. Values varied based on factors such as DO concentrations, temperature, and pH.

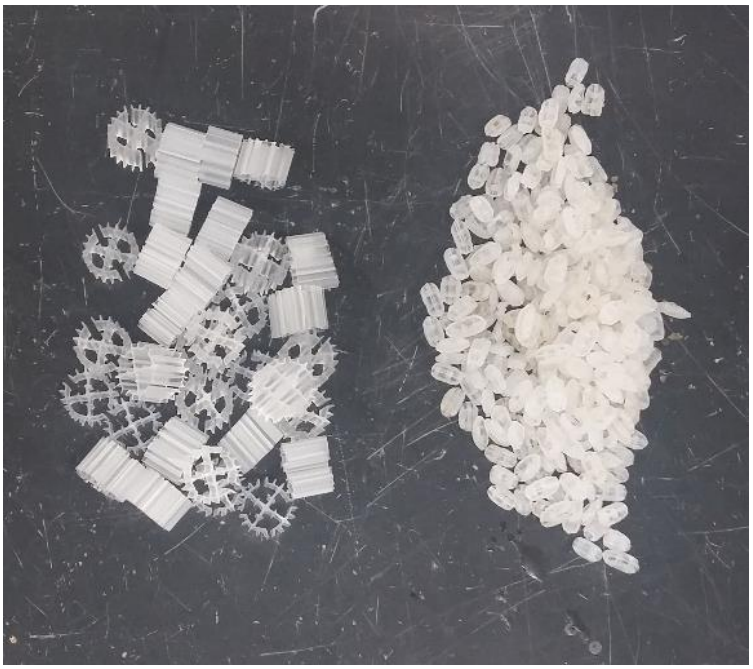

Figure 3: KMT (left) and EN (right) are commonly used in fixed-film submerged systems.

Nevertheless, comparative studies suggest that KMT may not be the most effective media for TAN removal, bacterial production, and overall nitrification in aquaculture systems (Bellelo, 2006; Pfeiffer and Riche, 2011). More recently, Aquaculture Systems Technologies, LLC. developed the Curler Advance X-1 with a configuration comparable to the KMT, but it is more appropriate for aquaculture applications (AST, 2017). This media has a VTR of 605 g-N/m^3 (Ebeling and Timmons, 2006; Research Institute for Coastal Aquaculture, 2011). In addition, KMT media is not easily affordable (Haandel and Lubbe, 2012), which is why MBBR's remain unpopular in developing countries' aquaculture industries. While interest in MBBR's in aquaculture is growing among researchers, recent innovative studies still utilize variations of the KMT (or similar) media.

2.9.3. Rice hulls (RH)

Rice hulls (RH) are the husks that cover rice grains (See Figure 4). Rice processors typically remove this coating during processing rice. Hulls are thus considered waste material, and therefore cost analysts consider their production cost to be negligible (Pollard et al, 1992., McKay *et al.*, 1999). Rice hulls are used for numerous applications. Their ashes can be used as cement after incineration (Boateng and Skeete, 1990). Silica gel is produced from the hulls' ashes (Kamath and Proctor, 1998; Amick *et al.*, 1980). For water treatment, they are utilized in combination with green algae to purify water by removing heavy metals (Mashah and Champagne, 1993), due their great antioxidant properties (Lee *et al.*, 2006). These properties are associated with their silica-rich composition. Nevertheless, the water solubility must be considered when determining the life-cycle of rice hulls as filtration media.

Figure 4: RH have an elongated shape and a larger surface area than microbeads. The curvy morphology of the sheath allows for cross-flow, as well as internal and external bacteria growth.

RH have an “opening” within the husk that facilitates cross-flow, and enables bacteria to grow both outside and inside. They are relatively large, which makes them an adequate fit for 3-phase reactors (Malone, 2013) that usually require media 6-13 mm in size.

2.10. Film removal

The term “sloughing” refers to the removal of the waste as the film falls off via hydraulic shear. Removal can be a delicate and complex procedure (Zhang *et al.*, 1994) and it must be done in a timely manner. Indeed, because of the anaerobic activity, acid by-products from nitrification in the deeper layer lower the film’s pH, which can be fatal to the bacteria. In addition, if the film gets too thick, the tank water can no longer travel through the media and the increasing hydraulic shear will eventually tear the film loose.

In bead filters, the film undergoes both mechanical weakening (the CO_2 and CH_4 bubbles, as well as peak hydraulic shear lead the film to detach from the media) and biological weakening (bacteria in the meso layer suffer endogenous respiration and weaken the film as they “consume” the slime). Furthermore, mechanical abrasion from the beads moving against one another allow for short biofilm duration (Sastry *et al.*, 1999). Backwash frequency should vary based on feeding rate, and is typically expressed in days per week (delos Reyes and Lawson, 1996; Singh *et al.*, 1999). Golz *et al.* (2002) developed a protocol for washing bead biofilters and removing biofilms for this particular media.

In MBBR, however, continuous media abrasion combined with continuous hydraulic shear cause removal of the film. Aquaculture engineers should determine MBBR biofilm kinetics by balancing media erosion-causing abrasion and biofilm management (Lee *et al.*, 2006; Barwal and Chaudhary, 2014; Wessman and Johnson, 2006; Holan *et al.*, 2016; Cabral *et al.*, 2009).

Indeed, the hydraulic shear must occur after the biofilm has grown to optimal thickness to ensure effective bacterial activity.

CHAPTER 3. AN EXPERIMENTAL COMPARISON OF RICE HULLS AND A FLOATING PLASTIC BEAD IN A NITRIFYING MOVING BED REACTOR

3.1. Introduction

Recirculating aquaculture systems (RAS) filter and reuse water, thereby minimizing water use. RAS are more advantageous than pond aquaculture because they allow for more control regarding the water quality and subsequently optimize fish health. Yet, around the world, pond aquaculture has helped produce cheaper commodity food fish, due to lower production costs. Al Aji *et al.* (2012) predicted that pond aquaculture would continue to dominate in developing countries because they cannot afford RAS initial investments. Nevertheless, the same areas continue to experience increasing concerns regarding aquaculture-related discharges, potentially harmful to the environment. For instance, ponds have contributed to the destruction of several coastal areas (van Wesenbeeck *et al.*, 2015; Saengrungruang and Boyd, 2014). Ponds systems are not water-efficient, as they can lose 20% of their water volume per day, especially in tropical countries (Bobstock *et al.*, 2010).

RAS are designed to provide clarification, biofiltration, circulation, aeration, and degassing for the tank water. A clarifier separates the solids via physical filtration. A biofilter promotes bacterial activity to remove toxic nitrogen via nitrification activity. Circulation can be performed through numerous devices, depending on the tank or pond configuration (Helfrich and Libey, 1991; Lazur *et al.*, 1997). Airlifts are adjustable, versatile tools (Parker and Suttle, 1987; Malone and Gudipati, 2005). Airlifts are all the more attractive options because of their ability to circulate, provide oxygen (aeration), and remove carbon dioxide (degassing) simultaneously (Loyless and Malone, 1998; Ridha and Cruz, 2001).

Several fixed film biofilters are engineered to operate with emerged media beds such as trickling filters and rotating biological contactors. Submerged fixed film systems require some form of media. For instance, floating bead filters (FBF's) are comprised of spherical or oblong plastic media that remains static except for intermittent expansion for backwash and biofilm management. Upflow sand filters utilize a sinking media. This characteristic is countered by the upward flow and intermittent expansion. Moving bed biological reactors (MBBR's) traditionally utilize plastic cylindrical media. This low-density bed is in constant motion or expansion, which promotes abrasion and subsequent biofilm management. A re-engineering of these aforementioned carriers and filter systems would manipulate floating/sinking characteristics in non-typically associated filter designs to assess the impact of the carrier itself, rather than the system. Pond and cage farming remain dominant in developing countries. As fish producers, developing countries seek to stabilize and expand their production. RAS are growing in use throughout Asia (Kumar *et al.*, 2010; Fadhil, 2012; Hoang *et al.*, 2017; Bobstock *et al.*, 2010), and media substitution and energy-efficient configurations could help implement RAS worldwide.

Although Asia dominates world rice production, rice is a common crop in other parts of the developing world such as Sub-Saharan Africa and South America (International Rice Research Institute, 2017). Rice by-product, rice hulls (RH) have an oblong shape and structure (external and inward ridged surfaces) comparable to the floating plastic beads used in PolyGeyser® filters (Wagener, 2003; Aquaculture Systems Technology, 2017). Their shape appears to be conducive to biofilm protection, and a large surface area appears to support bacterial biofilms. RH thus constitute a media potentially appropriate for a 3-phase reactor (air, water, and RH media). RH is a sinking material, but this characteristic is compensated by its low

bulk density that could facilitate aeration with less air volume (cfm). This would help lower horsepower and energy costs. This dissertation chapter seeks to contribute to scientific support to assist developing markets in need of scientifically proven solution that utilize local resources: rice hulls (RH).

3.2.Objectives

The overall goal is to engineer and design a biofilter to utilize RH as a biocarrier, along with a design that utilizes EN as a biocarrier. The EN-mediated apparatus is configured to operate in a manner similar to the RH biofilter, with a few modifications. The similar designs aim to focus primarily on media performance.

The study first seeks to develop design guidelines for these 3-phase reactors. Secondly, the RH nitrification capacity is measured in comparison with EN, a media well documented and which TAN removal capacity has been previously assessed within the aquaculture engineering community. The objective of the measurements is to develop volumetric TAN conversion rate (VTR) ratios for both media. Additionally, while EN have a lifetime value, the experiment seeks to determine the useful life of rice hulls. Finally, comparing data collected for both media will help assess whether a RH bioreactor (RHBR) is a viable option, and whether such an initiative is worth pursuing on a larger scale.

3.3. Background

Biofiltration in RAS is designed to take place as suspended growth or by growing a biofilm onto a structured media. Suspended growth systems are common in wastewater treatment. In aquaculture, Gutierrez-Wing and Malone (2006) suggested that they are appropriate for freshwater production, but need to be further studied and evaluated. Indeed, these systems provide a habitat where stock is more manageable than in ponds, and their operation does not

require the use of expensive plastic media (Hargreaves, 2006; Saliling *et al.*, 2007). Recent innovations in suspended growth systems include photosynthetic management of algae to control phytoplankton uptake, in combination with mechanical aeration and circulation (Hargreaves, 2006). Crab *et al.* (2012) advocated the use of biofloc technology whereby carbon input is increased directly into the water or through carbon-rich fish feed. This strategy enhances suspended growth systems by promoting nitrogen uptake through bacterial growth to complement nitrification (Schneider *et al.*, 2006; Crab *et al.*, 2012). Nevertheless, water quality is not easily controllable, due to the fragile balance between fish excretion and bacterial conversion. For this reason, fixed film systems are favored over suspended growth systems among many aquaculturists.

Fixed film biocarriers differ based on biofilter configuration (Malone and Pfeiffer, 2006). Trickling filters (TF) are emerged RAS reactors that immobilize or "fix" the bacteria on a high surface media, as smaller media would tend to clog the system. TF media have a relatively small specific surface area, and therefore TF exhibit a relatively low VTR. They also require high maintenance, and have high construction costs. Media substitution studies are still under study to optimize TF TAN removal (Harwanto *et al.*, 2011). Other fixed film emerged biocarriers include rotating biological contactors (Brazil, 2006). These rotating shafts are often combined with other biofilters (Davie, 1987; Timmons, 1993; Aurelio, 1996). Submerged packed biocarriers like submerged rock, plastic beds, and shell filters are more adequate for low loading applications, but can be modified to accommodate heavier loadings (Kumar *et al.*, 2009; Kumar *et al.*, 2011). Though they are relatively inexpensive, biofilm management can be complex, since there is no sloughing nor abrasion (Khademikia and Godini, 2016).

Expandable submerged filters include upflow sand, foam, and floating bead filters. Their static media bed is intermittently moved ("expanded") mechanically, hydraulically, or pneumatically. Floating bead filters (FBF) are submerged filters with low density media that complete the double task of capturing solids and biofiltering (Malone *et al.*, 1993). FBF's function with smaller biocarriers such as EN (see Figure 3). FBF's can also be used as clarifiers in combination with rotating biological contactors or other biofilters (Aurelio, 1996).

Expanded biofilters are also submerged, yet their granular media are continually mixed ("expanded") hydraulically, mechanically, or pneumatically. Microbead filters feature cell sections, each furnished with polystyrene beads as media (Timmons, 2003; Timmons *et al.*, 2006). Fluidized sand beds operate with an inexpensive, high surface area sand media (Summerfelt, 1996, 2006). Moving bed biological reactors (MBBR's) feature a biocarrier (typically Kaldnes-1 cylindrical "KMT" media) that abrades continually. Research with MBBR's is relatively new, but promising. Presently, MBBR media studies consider similar plastic cylinders. Although cylinder size and surface area have been evaluated as nitrification parameters (Ødegaard *et al.*, 2003; Wessman *et al.*, 2004; Colt *et al.*, 2006; Rusten *et al.*, 2006; Sen *et al.*, 2006; Pfeiffer and Wills, 2011; Khan *et al.*, 2014; Elliott *et al.*, 2017), media substitution is yet to be fully investigated.

EN media refers to the modified version the standard FBF spherical polyethylene, low-density media, as marketed by Aquaculture Systems Technologies in New Orleans, Louisiana. These innovative carriers are 3-5 mm thick oval beads (Malone *et al.*, 1993) that feature indents and a keel increasing bed porosity while providing biofilm protection even with frequent backwashing events, or during aggressive backwashing (Golz, 1997; Sastry *et al.*, 1999). Wagener (2003) measured the beads to have a density of 900 kg/m^3. Because beads are usually

used as a bioclarifier in a fixed packed bed, early FBF's required the use of supplemental nitrification strategies for high feed rates (Malone *et al.*, 1993). More recently, Guerdat (2010) measured VTR for different RAS, and determined that the EN-mediated system (PolyGeyser®) had better TAN removal rates than the KMT-mediated system across loading levels. Thus, EN beads constitute a standard of quality and efficiency for water treatment studies.

EN media and the modern KMT (KMT2) provide protection to their biofilms, thanks to their intricate shapes. Nevertheless, EN beads provide both interior and exterior biofilm protection, while MBBR's are sized according to interior SSA only (Rusten *et al.*, 2006). Both will develop a significant biofilm when operated in a packed bed with intermittent backwashing (abrasion). The EN media has a total specific surface area of 1100 to 1250 m^2/m^3, which is higher than the KMT's (Kaldnes-1) total specific surface area of 690 m^2/m^3 (Wagener, 2003; Ødegaard *et al.*, 2000)[3].

3.4. Methods and materials

A three-phase (liquid, gas, and solid) reactor similar to a MBBR was designed utilizing RH as biocarrier. Water velocity (liquid phase) allowed for transport and sludge suspension. Air injection (gas phase) provided aeration and diffusion. Additionally, airlifts allowed for media circulation and water recirculation. The RH media (solid phase) constituted the biocarrier for biofilm growth (Sánchez and Matsumoto, 2012). The rice hull bioreactor (RHBR) contained an internal sludge capture basin from where the sludge was evacuated through a pipe.

Three identical prototypes were evaluated against three identically modified prototypes using EN media. Each reactor was connected to aquarium tanks hosting killfish. High protein

[3] In practice, values can be expected to be 300-350 m^2/m^3 (Rusten *et al.*, 1994; Rusten *et al.*, 2006).

fish feed was increased 8 times in 1 g increments to produced increasing loading within ultra-oligotrophic to mesotrophic levels, and filter water was collected for 9 data points. Samples were tested for nitrite, ammonia, and pH. Results were compiled in a spreadsheet and statistically analyzed.

3.4.1. System design

According to findings from previous studies (Malone and Beecher, 2000; Drennan *et al.*, 2006), EN beads have a nitrogen removal capacity (VTR) of approximately 750 mg-N per L of beads/day. A more conservative VTR of 700 mg-N was considered. The volume of beads (V_b) needed to remove 700 mg-N was calculated as the quotient of loading over VTR, that is, 1 L. With an experimentally determined hull ratio or 4:1, the reactors contained 3 L water and 1 L media. The nomenclature for the reactor's design calculations, which are detailed in the following step-by-step equations, is detailed in APPENDIX A.

Neglecting transport, the bioreactor can be sized by equating the nitrogen load generated by the feed F (in kg/day) to the biofilter's conversion rate. The mass loading (M_L) is calculated from the excretion constant times the feed times the protein adjustment factor, P_{adj}.

Loading (M_L) = Conversion

$$M_L = F \times E_{TAN} \times P_{adj}$$

The excretion constant (E_{TAN}) known to be 30 g-N/kg of feed-day (Randall and Wright, 1987).

Given the peaked total fingerling, mass of 0.45 kg /tank and a body feed rate of 4% of body weight the overall feed load F is calculated as:

$$F = \frac{0.45\ \text{kg}}{\text{tank}} \times \frac{0.04\ \text{kg feed/day}}{\text{kg-fish}} = \frac{0.018\ \text{kg feed}}{\text{tank/day}}$$

Substituting F and E_{TAN} to the loading equation, a load of 617 mg-N/day is obtained. To calculate the filter size for an assumed amount of loading, the beads' volume V_b in the filter was calculated using the empirical 4:1 ratio for the hull ratio. V_b is directly proportional to the load divided by the EN VTR of 750 g-N/m^3-day. Thus,

$$M_L = \frac{18\text{ g feed}}{\text{day}} \times \frac{30\text{ g-N}}{\text{kg-day}} \times \frac{1\text{kg}}{1000\text{ g}} \text{x} \frac{50\%}{35\%} \text{x} \frac{1000\text{ mg}}{1\text{ g}} = \frac{770\text{ mg-N}}{\text{day}}$$

$$V_b = \frac{M_L}{VTR}$$

$$V_b = \frac{770\text{ mg} - \text{N}}{\text{day}} \times \frac{\text{Loading/day}}{750\text{ g/L} - \text{day}}$$

$$V_b = \frac{770}{750} \cong 1\text{ L of media}$$

By isolating the biofilter in a mass balance, the required recirculation flow can be computed under the assumption that the transport into the biofilter must equal the conversion. The flow rate Q_{TP} is calculated assuming a tank ammonia level of 0.5 mg/L. It is equal to the loading divided by the ammonia under the assumption the delivery must equal the conversion.

$$Q_{TP} = \frac{L}{A_t} = \frac{770\text{ mg-N}}{\text{day}} \times \frac{1}{0.5\text{ mg/L}} = \frac{1540\text{ L}}{\text{day}} \times \frac{1\text{ day}}{1440\text{ min}} = \frac{1\text{ L}}{\text{min}}$$

The tank's hydraulic retention time (HRT_T) is defined by the volume of the reactor (V) and the flow (Q_{TP}).

$$HRT = \frac{V_R}{Q_{TP}}$$

$$HRT_R = \frac{4\text{ L}}{1\text{ L/min}} = 4\text{ min}$$

The over flow velocity V_o is directly proportional to the flow rate divided by the clarifier's surface area A_c. V_o must be less than the V_s, which is the settling velocity of the rice hulls.

$$V_o \leq V_s$$

$$V_o = \frac{Q_{tp}}{A_c}$$

It is common practice for activated sludge to have a clarifier in the range of 400-1200 gpd /ft^2-day. The sinking velocity for a clean RHBR's V_{SRH} is as follows:

$$V_{SRH} = \frac{0.08\ \text{in}^2}{\text{sec}} \times \frac{60\ \text{sec}}{\text{min}} \times \frac{1440\ \text{min}}{\text{day}} \times \frac{\text{ft}^3}{1728\ \text{in}^3} \times \frac{7.48\ \text{gal}}{\text{ft}^3} \times \frac{144\ \text{in}^2}{\text{ft}^2} = \frac{4308\ \text{gal}}{\text{ft}^2/\text{day}}$$

Based on empirical observations for clean RH, the value was found to be (4290 gal/ft^2/day), which is consistent with $V_o \leq V_s$.

$$V_o = \frac{1\ \text{Lpm}}{12.75\ \text{in}^2} \times \frac{144\ \text{in}^2}{\text{ft}^2} \text{x}\ \frac{\text{gal}}{3.785\ \text{L}} = \frac{2.98\ \text{gpm}}{\text{ft}^2} \times \frac{1440\ \text{min}}{\text{day}} = \frac{4297\ \text{gal}}{\text{ft}^2/\text{day}}$$

On the other hand, since beads are a floating media, the EN 3-phase reactor does not require any overflow velocity adjustments. These calculations helped generate and refine 3-D drafts by Solid Works presented in Figure 5 and Figure 7. The reactors were built using ¼ in. thick acrylic and PVC pipes, connecting them with the 40 L glass aquarium tanks (more detailed drafts are presented in APPENDIX B, elaborating on specific internal and external dimensions).

Both EN and RH reactors were adapted to have a total capacity of 4 L, designed to host the same media volume, and to circulate at the same airflow rate of 563 Lpd. The water from the aquarium tank circulated to the reactor and back to the tank (throughput) at 0.32 Lpm, using airlift to control the circulation and the flow rate. As shown in Figure 5 and Figure 7, the media are expanded in the aeration zone (3) that contains an airstone (2).

For the EN prototype presented in Figure 5 and Figure 6, the accumulated sludge settles in a "dead spot" (8) where there are no beads. The sludge is hydraulically directed to the removable sludge pipe (7). As one pulls down the sludge pipe (7), the sludge is evacuated from the system. The reactor functions in a manner similar to a moving bed reactor. After a total retention time HRT_R of 4 min, the water returned to the aquarium tank. The air (2) inside the

reactor section expands the media between the inner back wall and the interior air stone, creating a completely mixed reactor, also called a continuous stirred tank reactor (CSTR). As the sludge settles in the clarifier (8), the design bottom wall angled at 45° helps avoid clogging and failure (settling). As the water returns to the tank (5), a sludge capture receptacle, lined with a polyester filter pad, serves as an additional capture device to filter any solids that were not evacuated via the sludge chamber (7). Figure 6 shows the relationship between the EN3-P and the tank.

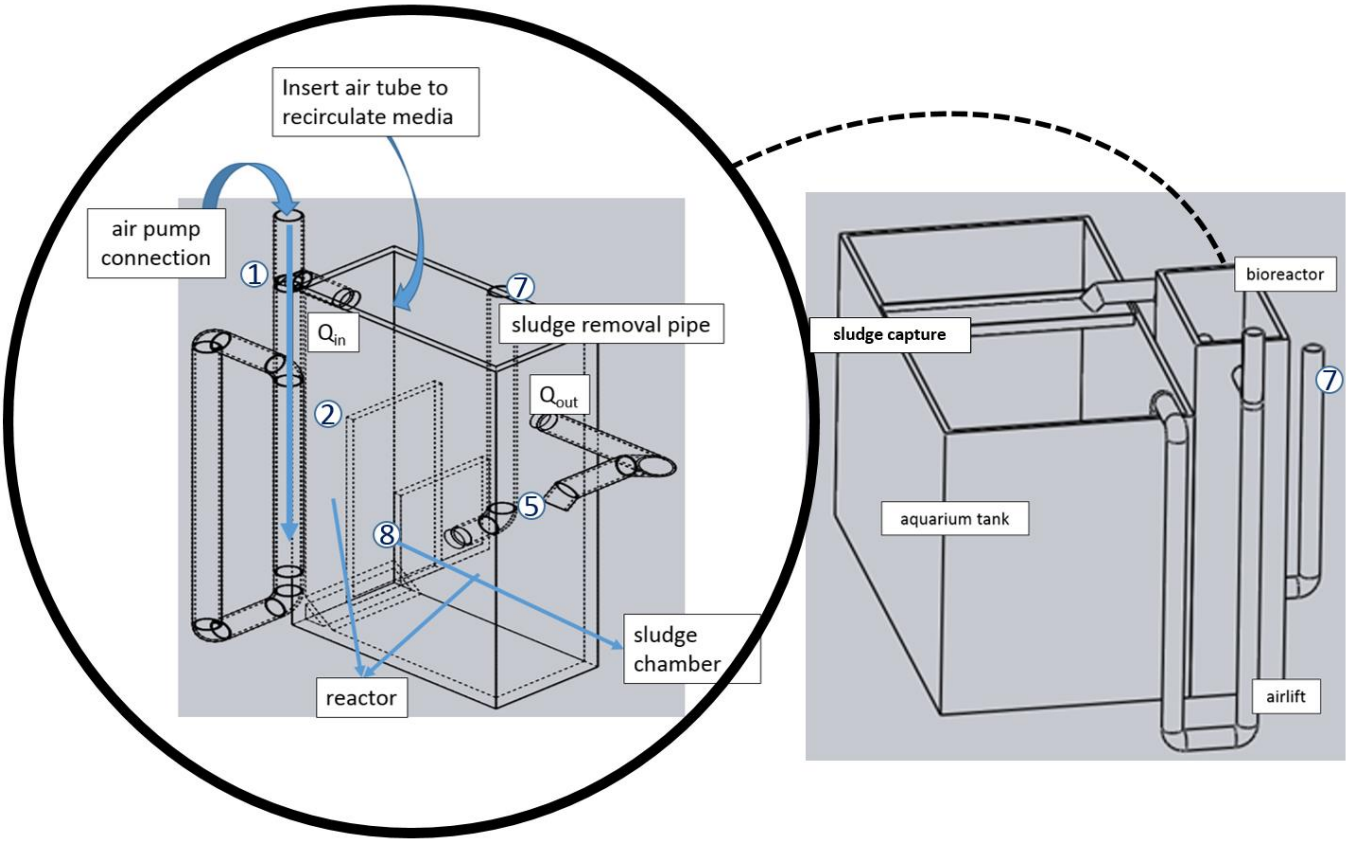

Figure 5: Complete apparatus connecting EN reactor to aquarium tank with enlarged view of the reactor

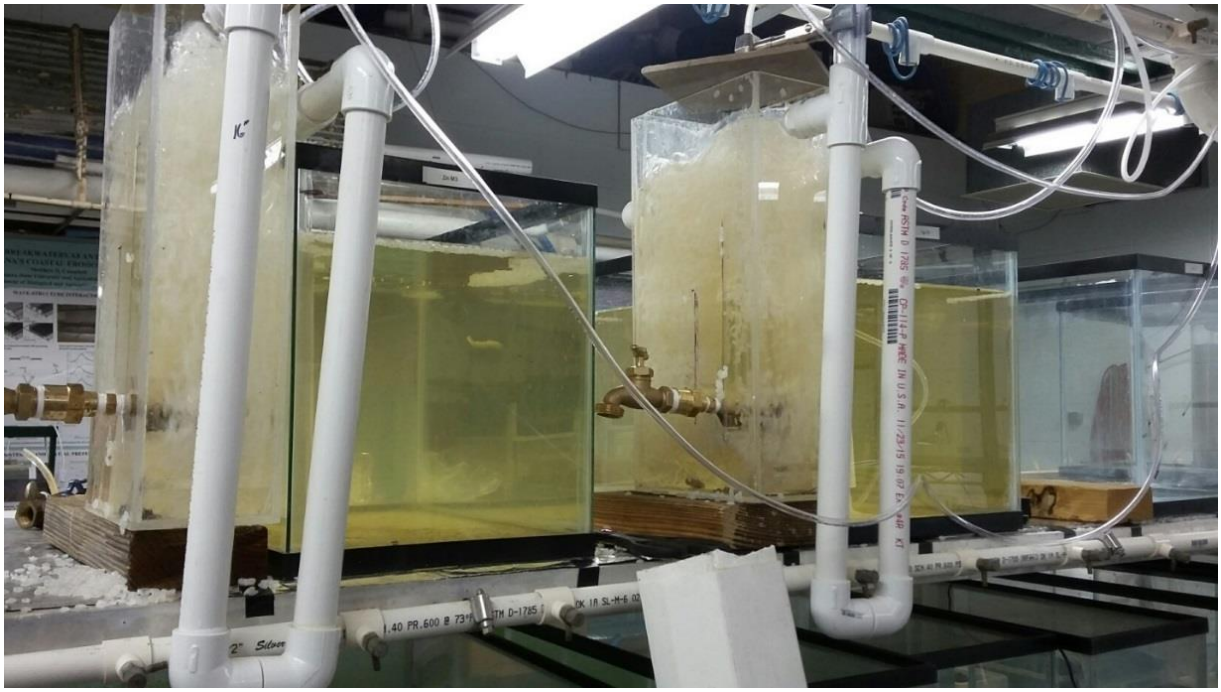

Figure 6: Two EN reactors in laboratory setting

The RH design presented in Figure 7 and Figure 8 utilizes RH a sinking media. For this reason, clarification was addressed differently for the RH prototype. The media circulates continually between the airlift pocket (3) and the reactor area (2). The airlift's flow (1) controls the solids' recirculation rate from the tank. Indeed,,the rising current drags the flocculent particles of waste upwards into the clarifier area (4), as the media sinks (3). The suspended waste is then evacuated through Q_{out} (5) and lands on a receptacle bed (6) lined with a plain polyester filter pad of 254 × 15 cm. Q_{out} also removes the broken-down RH as sludge upon depletion.

The RHBR also functions similarly to a moving bed reactor. The water flow rate was established to allow 4 min. retention time before returning to the aquarium tank. It also operates as a CSTR, as the air tube placed inside the aeration section (2) recirculates the media in this aeration zone. The angled bottom wall is more pronounced in the RHBR than in the EN reactor,

since RH is a sinking media. Instead, the hulls slide downward and then the airlift (2) circulates them around.

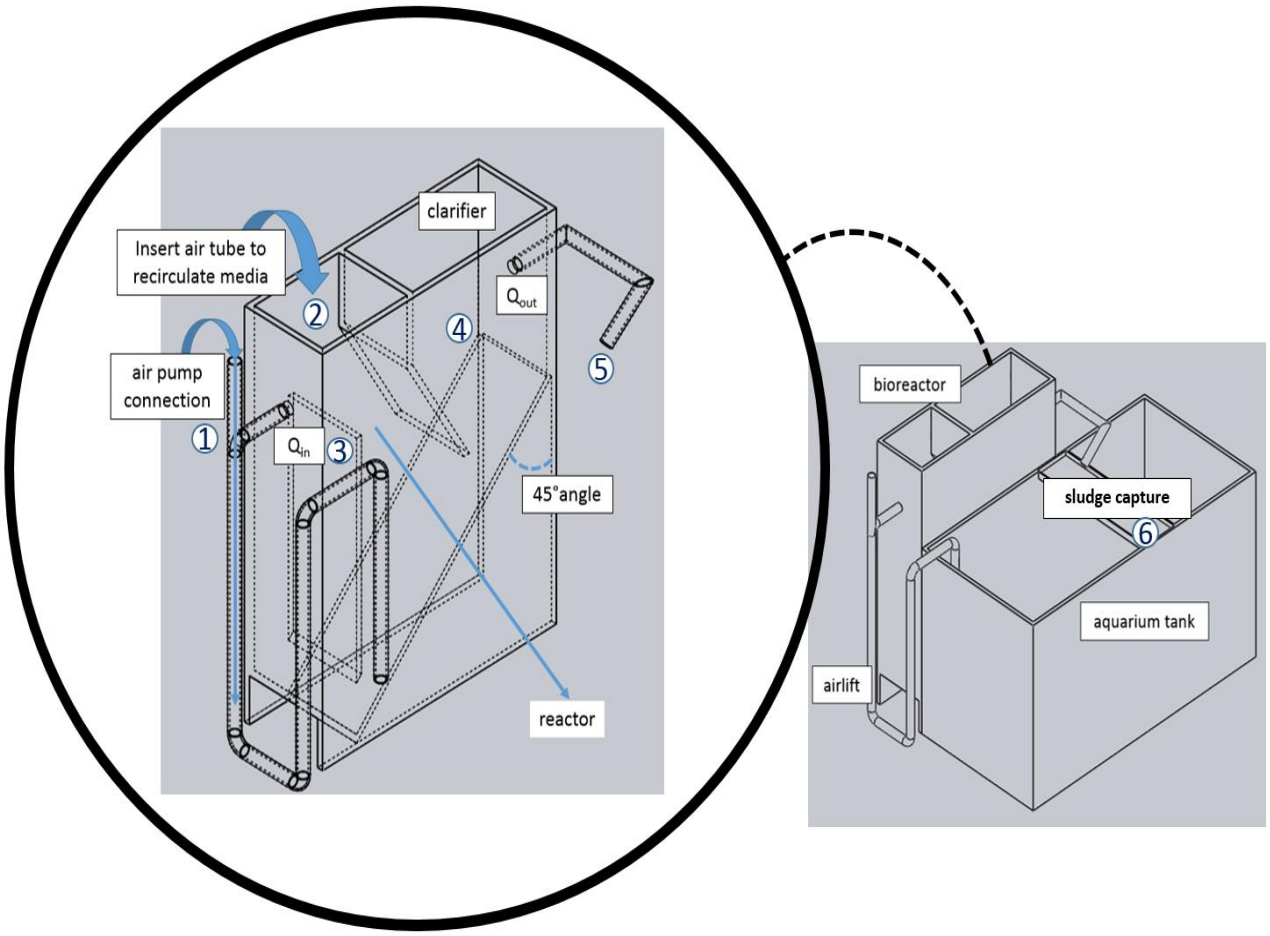

Figure 7: Complete apparatus connecting RH reactor to aquarium tank with enlarged view of the reactor

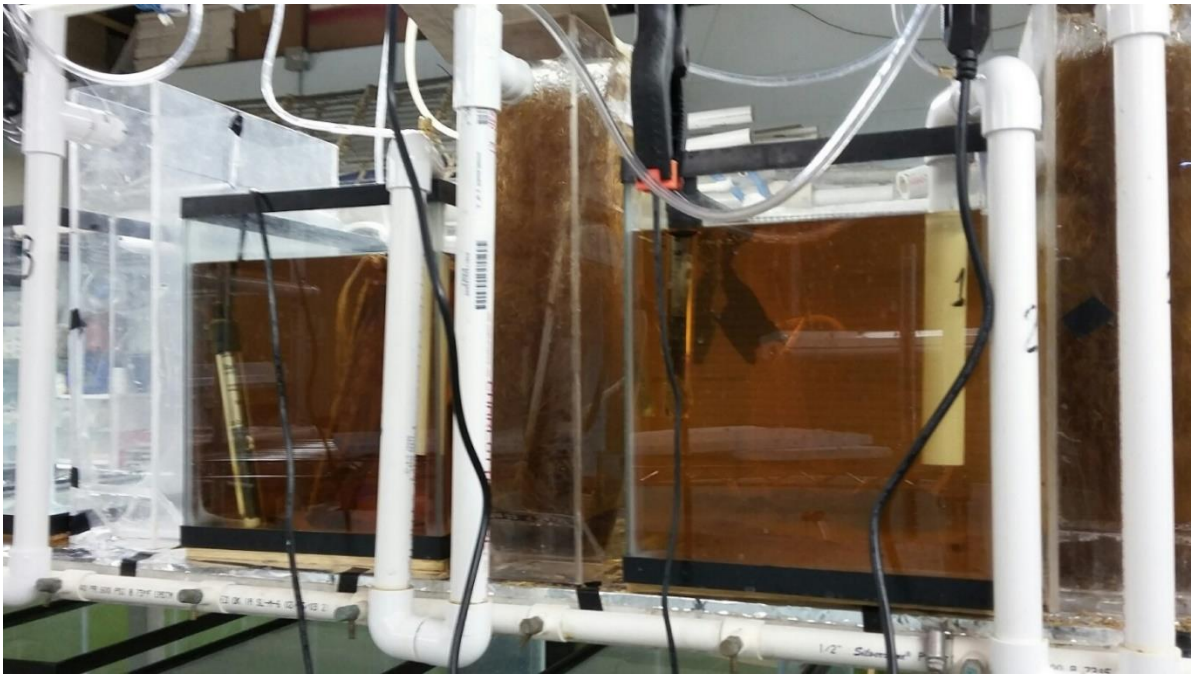

Figure 8: Two RH reactors in laboratory setting

3.4.1.1. Criteria and rationale for sizing within the reactor design

3.4.1.1.1. Media flotation and sinking velocity

The RH sink rate is a preliminary measurement that is indispensable. Not only was it needed for comparative purposes with EN media (Figure 9), but it was also necessary in order to ensure that it remained more than the sludge sink rate. Indeed, the only way to remove sludge was to design a reactor where the RH's sinking rate was longer than an activated sludge biofloc particle. The velocity of settling and hydraulic regime that would push out the sludge but not the RH, out through the clarifier determined the design criteria: the sinking velocity of the RH V_{SRH} and the overflow rate of the clarifier V_o (see APPENDIX A). To that end, a 189.25 L (50 gal.) drum was initially used to calculate the velocity.

Figure 9: EN and RH have similar lengths but different SSA. They also differ in density, as EN beads float, while RH is a sinking media.

Twenty randomly chosen pieces of EN beads were held to remain in the drum of water at a depth of 30.48 cm before release, each time for 20 reps. Flotation velocity for each rep was measured and averaged.

The RH were soaked for three days to allow proper sinking velocity. A 30.48 cm. clear cylinder was filled with water. Then, 20 pieces of RH were randomly chosen from the soak batch. The velocity of the RH was measured by dropping one piece at a time and recording the time it took to reach the bottom of the cylinder. The average of the 20 reps was recorded to be used as parameter of reference in the design of the RHBR's clarifier.

Sinking times averaged at 12 sec. for RH and flotation averaged at 10 sec. for EN. Corresponding velocities for each rep are presented in Figure 10. Average RH velocity is 2.6 cm/sec, and EN average velocity is 3.2 cm/sec.

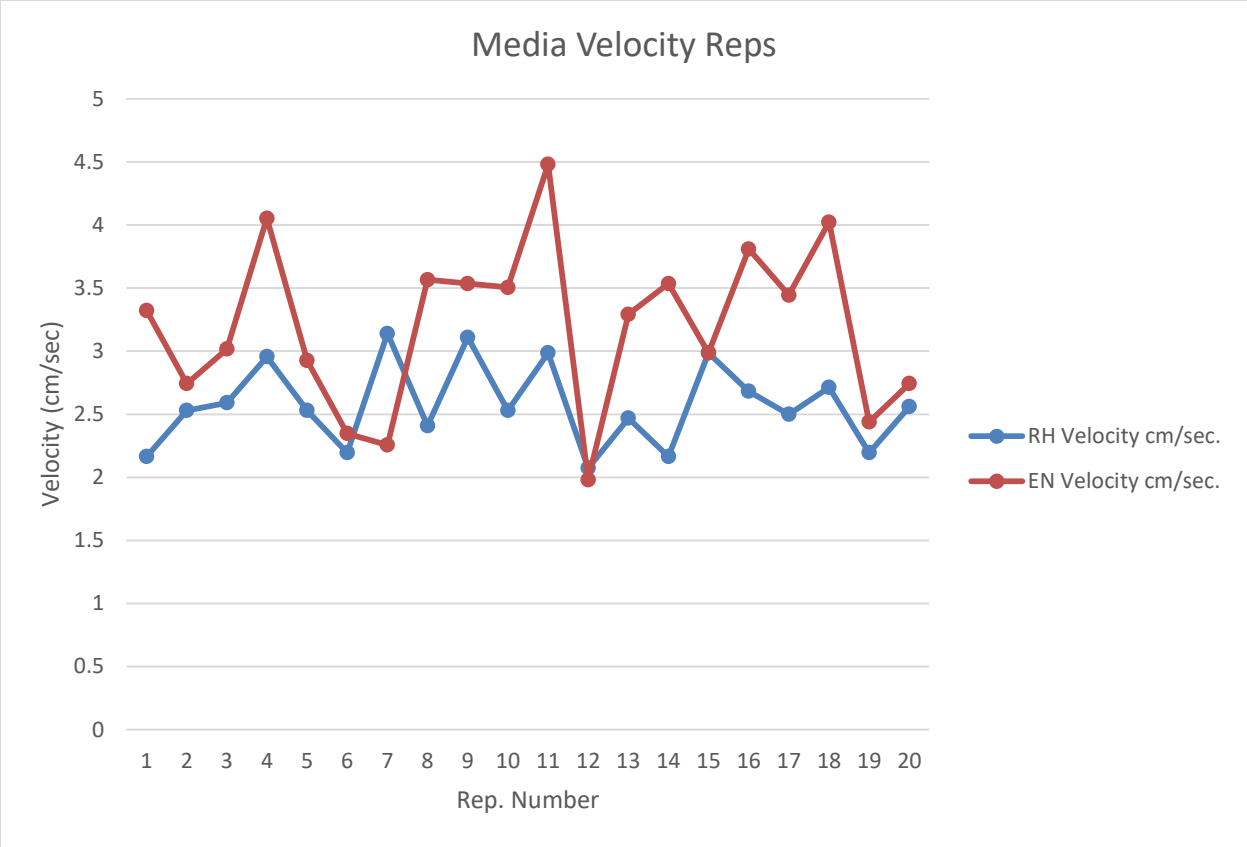

Figure 10: RH and EN media average sinking and floating velocity.

3.4.1.1.2. Sizing the clarifier

While Q_{tp} is the flow through the reactor based on its retention time HRT_R, Q_x refers to the critical flow in the reactor required to ensure circulation and removal. This rate helps calculate how big or small the clarifier needs to be. V_0 denotes the RH overflow rate, calculated to be 70 Lpd/m^2. The reactor's cross section area A_c is expressed as the quotient of the recirculation (Q_{tp}) and V_0, resulting in 148.5 cm^2. The clarifier's surface overflow rate (SOR) is the quotient of the recirculation rate Q_R^C and the ammonium concentration $(A)_c$, which is assumed to be 1 ppm-N.

The SOR was designed to be very conservative initially, as the nature of the flow was still experimental. After observing the lab units operate, an overflow rate of 229 Lpd/m^2 was used for the full-scale design. This value was more consistent with the rate suggested by Metcalf and Eddy's (2014) guidelines for extended aerated clarifier, which recommend a range of 70-280 Lpd/m^2 for the biological nutrient removal application described in this study.

3.4.1.1.3. Airlift sizing for the reactor

The airlift ratio was 4:1 (Gudipati, 2005). Out of the 38.1 cm long, 1.9 cm diameter PVC pipe, 30 cm were submerged (see Figure 8 and Figure 6). Each reactor is fed by its own external airlift that inject air into a column "PVC pipe" to lift and transport the water vertically into each reactor from the tank. While gravity brings water back to the tanks. The inject airflow rate was set at 0.44 lpm for each reactor.

3.4.2. Fish population

Loading generated from live fish was more appropriate than chemical feed (Davis *et al.*, 2011) because it offers a more realistic composition of organic and nitrogen compounds. These compounds are difficult to mimic chemically[4].

Although any marine species could be used, the *fundulus grandis* or cocahoe minnow (Figure 11) is a relatively abundant species of killfish in the Gulf region, so using it does not significantly present ecological challenges. In addition, the fact that Gulf killfish have a high tolerance for diseases and temporarily low DO concentrations (Anderson *et al.*, 2012) facilitates the humane treatment of the tested population. Cocahoe minnows live in both saltwater and

[4] Guerdat *et al.* (2010) reported that mimicking fish waste produces relatively unreliable results and stress the importance of using live fish when assessing a filter's TAN removal rate.

freshwater. Despite a preferred salinity of 1-3 ppt, Gulf killfish tolerate levels ranging from 0.5 to 76.1 ppt. A temperature of 28°C is an optimal thermal condition.

Figure 11: *Fundulus grandis* have a high tolerance for various water quality conditions. Killfish were purchased from Terry's Live Bait in Golden Meadow, Louisiana.

3.4.3. Media

Husks from locally grown long-grain, Jazzman rice developed at the LSU AgCenter, was selected as media[5]. Davis *et al.*, 2011 calculated its SSA to be 3707 m^2/m^3 (1130ft^2/ft^3). Nevertheless, microscope observations and calculations showed that biomass builds on half the available SSA, leading to a SSA of 1850 m^2/m^3 in practice. RH for virtually all rice types are

[5] Knud Thomsen calculations were completed. Results showed that, despite variations in shape, surface area over volume ratio is similar across rice types.

brown, oblong, biodegradable husks (see Figure 12). With a low bulk density of 150-400 kg/m^3, they measure an average of 6.5 to 10 mm in length, 4 to 6 mm in width (or circumference, when fully open), and 1.5 mm in thickness (Temitope *et al.,* 2015; ricehusk.com, 2017)**.** They are slightly rugose to the touch, making them appropriate for bacteria to attach and grow (Pons *et al.*, 2011; Yoda *et al.*, 2014). . Sergienko *et al.* (2004) measured the dry, organic composition of rice hulls to be, in terms of weight: 39.8-41.1% carbon, 5.7-6.1% hydrogen, 0.5-0.6% oxygen, and 37.4-36.6% nitrogen. RH are essentially made of silica (silicon dioxide or SiO_2). Koz'mina (1976) measured the ash, inorganic content to be 93.4% SiO_2, 0.05% Al_2O_3, 0.06% Fe_2O_3, 0.31% CaO, 0.35% MgO, 1.4% K_2O, 0.1% Na_2O, and 0.8% P_2O_5

Figure 12: RH's creases and abrasive surface constitute a favorable topography. Their interior and exterior surface area promotes bacteria growth.

3.4.3.1. Criteria and rationale for media sizing

MBBR guidelines were considered to size EN and RH media. Ebeling (2006) indicated that in MBBR's, the media typically takes up to 70% of the reactor's volume. Exceeding this amount would reduce the mixing efficiency. The media volume is typically defined by the volume of the reactor, so it is preferable to insert as much media as possible to get a high

conversion ratio. Nevertheless, space is needed for the media to move continually. Likewise, the water volume in the reactor is commensurate with media volume: the RH ratio should be around 4:1for efficiency reasons. Since the equivalency of RH and bead media was yet to be determined at first, we elected to use the same amount as beads in terms of volume. Nevertheless, the difference in the two media's surface areas (see Figure 9) and durability must be considered when seeking to establish equivalence. Media amounts were routinely adjusted based on our observations.

3.4.4. Experiment procedures

The lab-based experiment was scaled to six 4 L reactors, each connected to one 40 L aquarium tank. Three reactors contained RH media, and three contained EN media. Stock water was prepared by filling a 4000-L tank with tap water and allowing to sit for at least 5 days to remove any forms of chlorine that could be harmful to the killfish. Experimental tanks were filled with 40 L salt stock water and populated with killfish acquired from a local bait shop. The salt water was mix to the require salinity in parts per thousand (ppt). Hauling and handling was completed based on the guidelines and protocols detailed in Anderson *et al.* (2012). At 10-day intervals for the duration of the experiment, tanks were reduced to 75-80% of original fill by either evaporation or manual removal, and stock water was added to return volume to 40L. Water samples were collected daily to monitor initial acclimation. Temperature was set a 30°C initially, to speed up acclimation (Saidu, 2009). Both EN and RH reactors took 3 weeks to acclimate initially, and temperature was then decreased to 28°C. A two month period was allowed for stabilization of the system and to allow killfish to adjust to conditions before measurements were taken.

In order to achieve specific TAN from excretion rate, the feed rate was progressively increased. The design amount of fish per tank at peak level was set at 24 fish, each weighing approximately 10 to 18 g. Thus, in practice, tanks initially start 4 fish, each weighing 7 to 9 g, with a daily feed rate of 1 g of high protein feed per g of fish. The Otohime feed from Reed Mariculture Inc. ensured that the fish maintained a rich, high protein diet. Average loading was calculated as the product of feed, nitrogen excretion rate, and protein ratio and the design's corresponding loading was determined to be 700 mg-N/m^3-day. pH was maintained at 7.2-7.8. A salinity meter and a pH meter were used to monitor pH and salinity twice daily. Sodium bicarbonate (baking soda) served as a buffer to regulate and control pH. Water was kept at 28ºC, salinity at 3.30 ppt, and pH between 7.2 and 7.8.To address water loss due to evaporation and splashes, tanks were replenished every 10 days by adding 1/5 of each tank's volume. Table 1 summarizes fish treatment and management.

TAN was increased by increments, by adding more fish and more feed, in order to assess the biofilter's efficiency at TAN removal for different trophic levels. Trophic level classification listed in Table 2 refers to Malone and Pfeiffer's (2006) classification for specified aquaculture applications. Thus, feed amounts were increased by 1 g, each new increment followed by an acclimation period of 2 to 3 days for the EN reactor and 5-7 days for the RHBR. Upon acclimation, samples were collected. Fish weight per tank was adjusted correspondingly, in approximately 200 g increments, as detailed in Table 2. Loading was calculated from daily feed, according to the following formula:

$$\text{Estimated loading} = \frac{g}{day} \times \left(\frac{30g - N}{1\,kg}\right)\left(\frac{50\%}{35\%}\right)\left(\frac{Kg}{1000g}\right)$$

Table 1: Summary of the management plan from beginning to end of the experiment

Item	**Amount/ dose**	**Purpose**	**Frequency**
Gulf killish (Cocahoe minnow, *fundulus grandis*)	24-132 fish 4-22/tank	Test population	Replaced upon fish death
Bi-carbonate	0.11 kg/kg of feed	pH control	As needed to regulate pH
Feed (Otohime Fish Diet)	0.5-4.5 g/ tank/day	High protein feeding	2 times a day
Media	1 L	Nitrification/filtration	EN: once
			RH: replenished every 18 days
Water	357.72 L	Fish environment + filtration	Replace 20% to the tank every 10 days

Table 2: Feeding and fish replenishment plan

Feed/tank/day (g)	**Fish/tank (g)**	**Loading (g-N/g_{feed}/day)**	**Trophic level**
1	34	0.043	Ultra-oligotrophic
2	80	0.086	
3	126	0.129	Oligotrophic
4	154	0.171	
5	172.5	0.214	
6	252	0.257	
7	300	0.300	Mesotrophic
8	352	0.343	
9	412.5	0.386	

3.4.5. Data collection and processing

Upon acclimation, water samples were collected from in the tank. Contents were measured with an Api test kit for concentrations of nitrite and ammonia. Samples were collected from each tank for 3 consecutive days, including the control tank. Data points were determined after reaching steady-state. The 3 data points corresponding to the 3 consecutive days of data collection for each tank were averaged.

Results were entered in an Excel® spreadsheet. A SAS software was used to complete the statistical analysis by showing the significant difference between EN and RH of p-value at point 5. An Excel® spreadsheet was created to generate graphs and a regression study. Ammonia and Nitrite concentrations were converted from percentages to mg/l, and added to the Excel® database. Statistical analysis was performed using SAS (version 9.3, Raleigh, NC) whereby loading treatments were separated according removal capability using Student's t-test.

3.5.Results

3.5.1. Observations

Initially, the fish in the RH system appeared healthy as the fish in the EN system. Two brief outbreaks of *Mycobacterium* infection occurred during the initial two-month adjustment period, after which the fish began to grow and procreate abundantly, and populations stabilized. Fish appeared visually healthy with overall good countenance. At the end of the experiment, each tank contained approximately 22 fish, each weighing approximately 12 g.

As the experiment progressed, the RH system exhibited more and more stability in terms of conversion. It also acclimated faster as the experiment progressed over time. At feed amounts 1-5 g/day, acclimation took 6 to 7 days. By feed amount 6 g/day, acclimation took 5 days. The

EN system took 3 days to acclimate at feed amounts 1-5 g. Then acclimation lasted 2 to 3 days at 6 g/day.

Water in RH-filtered tanks was darker than water in the EN-filtered tanks, which had a yellowish color. The media and sludge poly-filter were included in both the RH and the EN reactors' designs for consistency purposes. Yet, they were not needed at all for the EN reactors, as they displayed zero media loss. In this respect, the use of RH requires more timely and meticulous maintenance than EN reactors for two tasks: emptying the poly-filter, and replenishing the RH every 18 days. Nevertheless, pulling down the EN reactor's sludge pipe must be executed gently and with much caution, taking the flow rate into consideration, to ensure that the beads do not enter the pipe in the process. The frequency of manual removal depends on loading, as the sludge was visible through the clear acrylic pipe.

While EN has lifelong durability, RH depleted over time. Identical initial volumes were set for both media, and an adjustment plan was elaborated, based on observations and replacement needs. The hulls started to breakdown at days 18-20 of the experiment. By day 23-24, depletion of the RH media was complete. When depleted, all media was evacuated as sludge. Media loss was solved by adding 0.33 L (⅓ initial media volume). RH by day 18, for 3 consecutive days, according to the following dosage:

$$\text{Volume of replacement media} = \frac{reactor\ volume}{3} \times 3\ days$$

The procedure was repeated every 18 days, as summarized in the schedule presented in Figure 13:

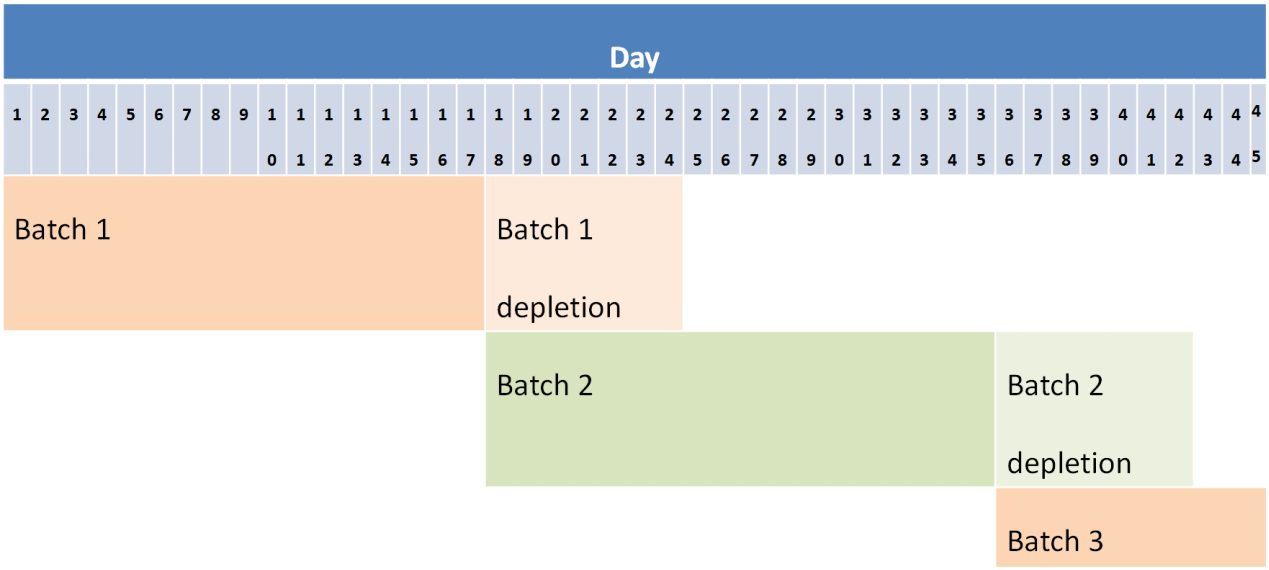

Figure 13: RH media replacement schedule

The temperature was regulated by a heater placed inside the aquarium tank. Daily temperature measurements were recorded and reported in Figure 14. The graph indicates high temperatures at first because of the higher settings established during the prior acclimation period (Saidu, 2009). The following fluctuations from the 28°C setting correspond to changes in the lab building's air and ventilation system. These fluctuations did not impact fish behavior, leading to the conclusion that the system can tolerate at least a 0.6°C increase, depending on the species.

Likewise, pH was measured daily, and maintained at 7.8. A drop in pH was noticed after each change in loading (feed and fish amount). This phenomenon is in line with the literature, as broken down ammonia from added feed and excretions produces more hydrogen cations (Boumis, 2016). This was buffered by the addition of bi-carbonate. The days with low spikes in the graph presented in Figure 15 correspond to the days when the increments were implemented.

The surges correspond to the subsequent addition of bi-carbonate. The short drops in pH did not affect fish health of behavior.

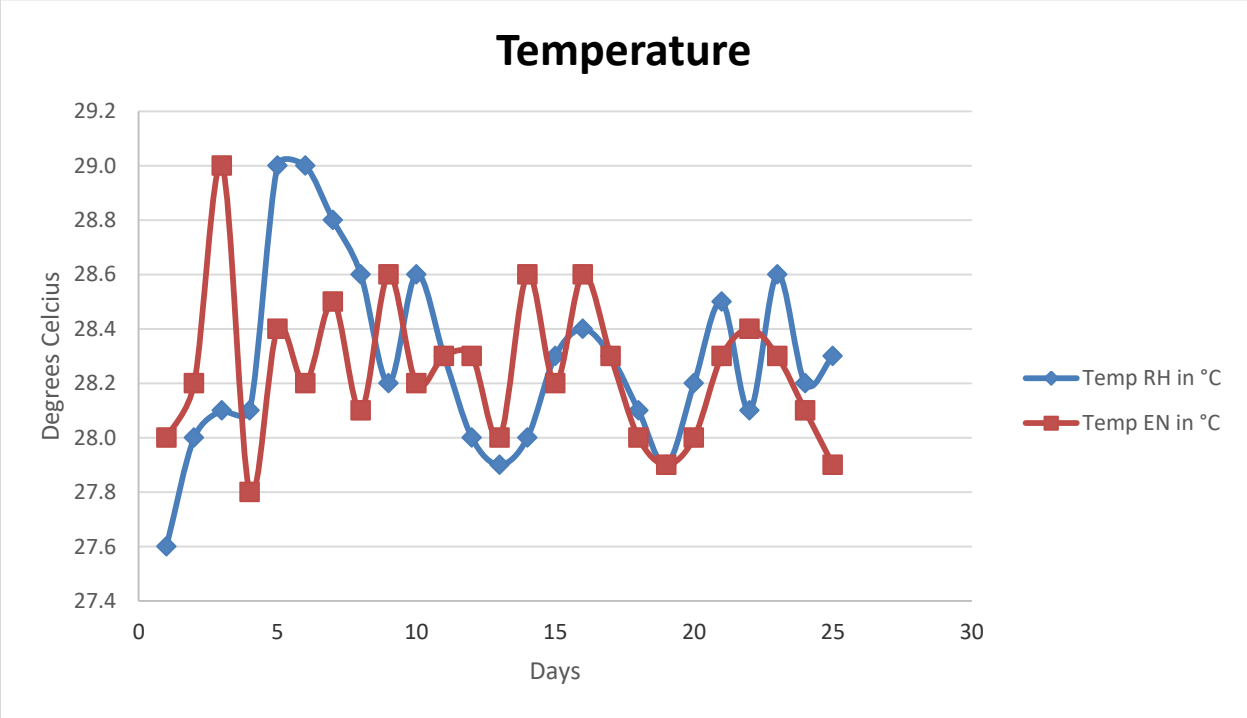

Figure 14: Daily temperature measurements

3.5.2. General results

Table 3 displays feed rate and loading parameters and their corresponding nitrogen concentrations at outflow for both EN and RH systems after acclimation. Each feed rate section in the table is expressed in 3 rows. As data were collected over 3 days and averaged, each value reported in the 3rd through 6th columns corresponds to the 3-day average for a given tank (3 RH-filtered tanks, 3 EN-filtered tanks). Since the control tank's reactor never reached acclimation to steady-state, results for the control tank were omitted.

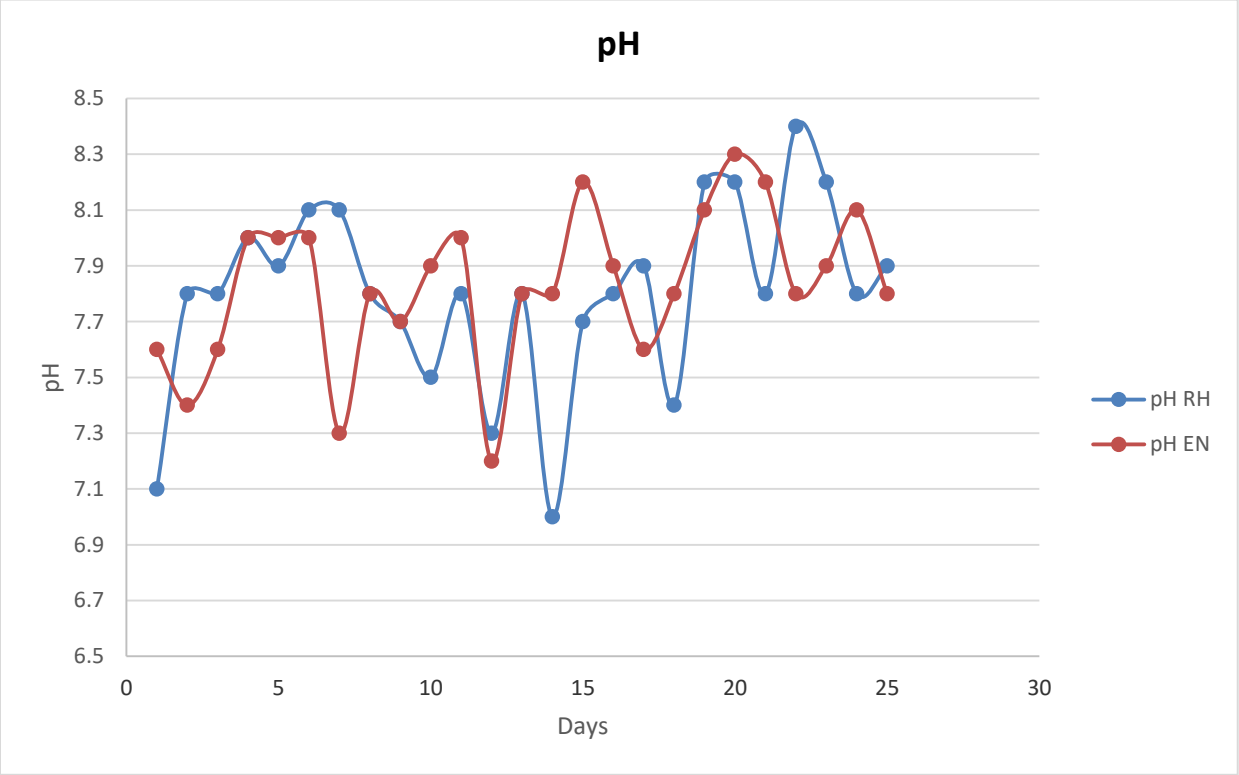

Figure 15: pH dropped after each feed and fish increase.

3.5.3. Ammonia

Ammonia measurements for each loading level are detailed in Table 3: Loading, concentrations of nitrogen and nitrogen removal rates are expressed as functions of the daily feed rate. At the ultra-oligotrophic level, RH and EN appear to have similar removal effectiveness. Slight variations with the difference between EN and RH's ammonia removal increases with higher loadings. Results indicate that EN beads are generally more effective at removing ammonia than RH for oligotrophic and mesotrophic applications.

NO_2 RH and NO_2 EN results correspond to nitrite concentrations in mg/L. NH_3 RH and NH_3 EN results correspond to TAN (total ammonia nitrogen) concentrations in mg/L.

Table 3: Loading, concentrations of nitrogen and nitrogen removal rates are expressed as functions of the daily feed rate.

Feed rate (g/tank/ day)	Loading rate (g-N/day)	NO_2 RH (mg/L)	NO_2 EN (mg/L)	NH_3 RH (mg/L)	NH_3 EN (mg/L)
1	0.043	0.045	0.030	0.060	0.050
		0.040	0.040	0.058	0.045
		0.040	0.025	0.052	0.040
2	0.086	0.047	0.050	0.094	0.090
		0.047	0.055	0.092	0.080
		0.040	0.030	0.086	0.080
3	0.129	0.053	0.058	0.115	0.120
		0.057	0.047	0.125	0.110
		0.054	0.048	0.127	0.115
4	0.171	0.058	0.060	0.165	0.108
		0.062	0.057	0.162	0.105
		0.064	0.058	0.166	0.110
5	0.214	0.100	0.104	0.200	0.165
		0.130	0.125	0.205	0.166
		0.180	0.110	0.195	0.158
6	0.257	0.170	0.120	0.230	0.155
		0.155	0.125	0.225	0.165
		0.170	0.115	0.232	0.155
7	0.300	0.175	0.120	0.275	0.214
		0.175	0.125	0.263	0.219
		0.180	0.115	0.259	0.205
8	0.343	0.260	0.170	0.325	0.259
		0.275	0.190	0.315	0.260
		0.255	0.135	0.350	0.258
9	0.386	0.305	0.195	0.360	0.300
		0.320	0.245	0.375	0.315
		0.335	0.245	0.395	0.305

The denotation τ is used to express TAN conversion as a function of media volume (Malone and Pfeiffer, 2006). τ indicates the effectiveness of the media in terms of TAN removal rate VTR (volumetric TAN removal rate), and equals the slope of the regression line on Figure 16, which used a forced zero-intercept to assume conversion at TAN=0.

The lab systems exhibited a mean VTR of 1219 mg-N/day-m^3 and 1025 mg-N/day-m^3 for EN and RH, respectively, once the systems had become most stable and fully in-situ. τ is quotient of TAN assumed at 1 mg/L. It is calculated from the following equations, adapted from Malone and Pfeiffer (2006):

$$VTR = \tau \times TAN$$

$$\tau = \frac{VTR}{TAN}$$

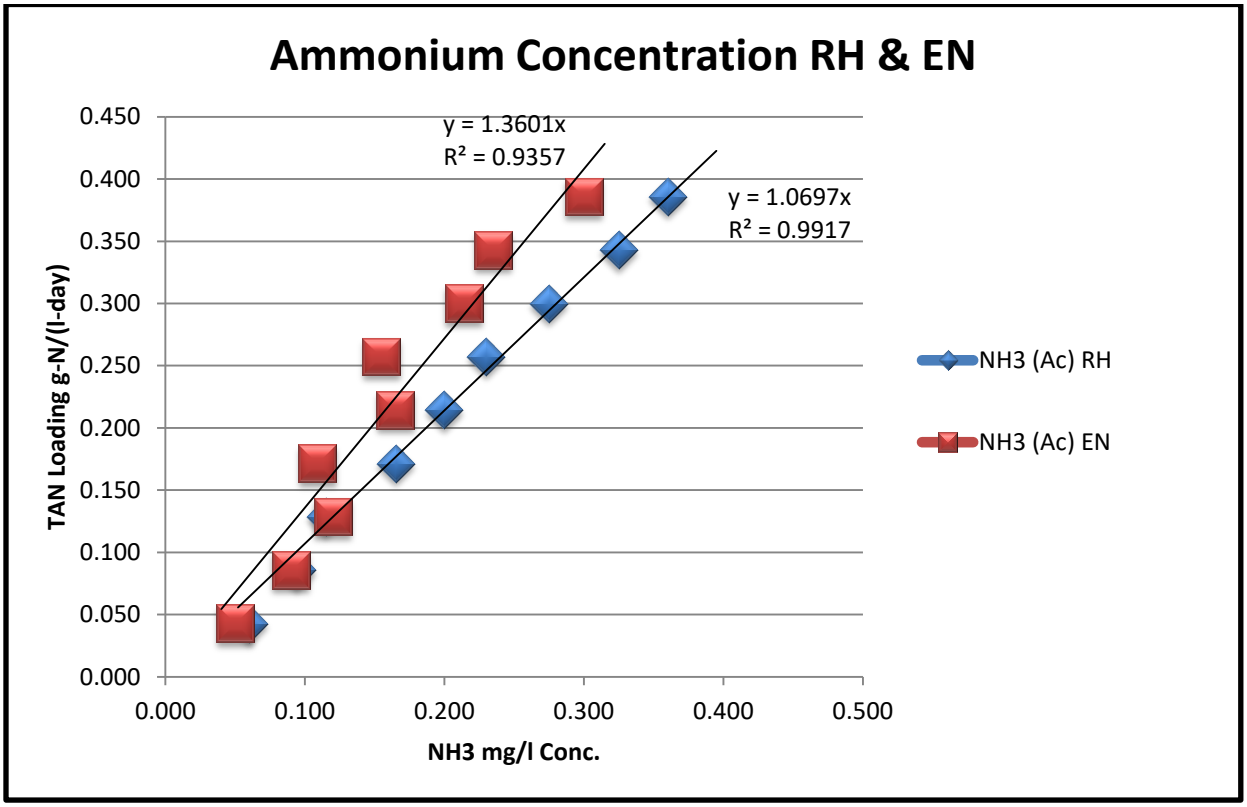

Figure 16: Ammonia concentrations for RH and EN based on loading at inflow

3.5.4. Nitrite

At ultra-oligotrophic and oligotrophic levels, nitrite removal is similar between RH and EN. RH nitrite concentrations are actually lower than EN at loading 0.08, 0.13, 0.17, and 1.22 g-N/L-day. In mesotrophic conditions, EN appears to be more effective at removing nitrite than RH, as illustrated in Figure 17.

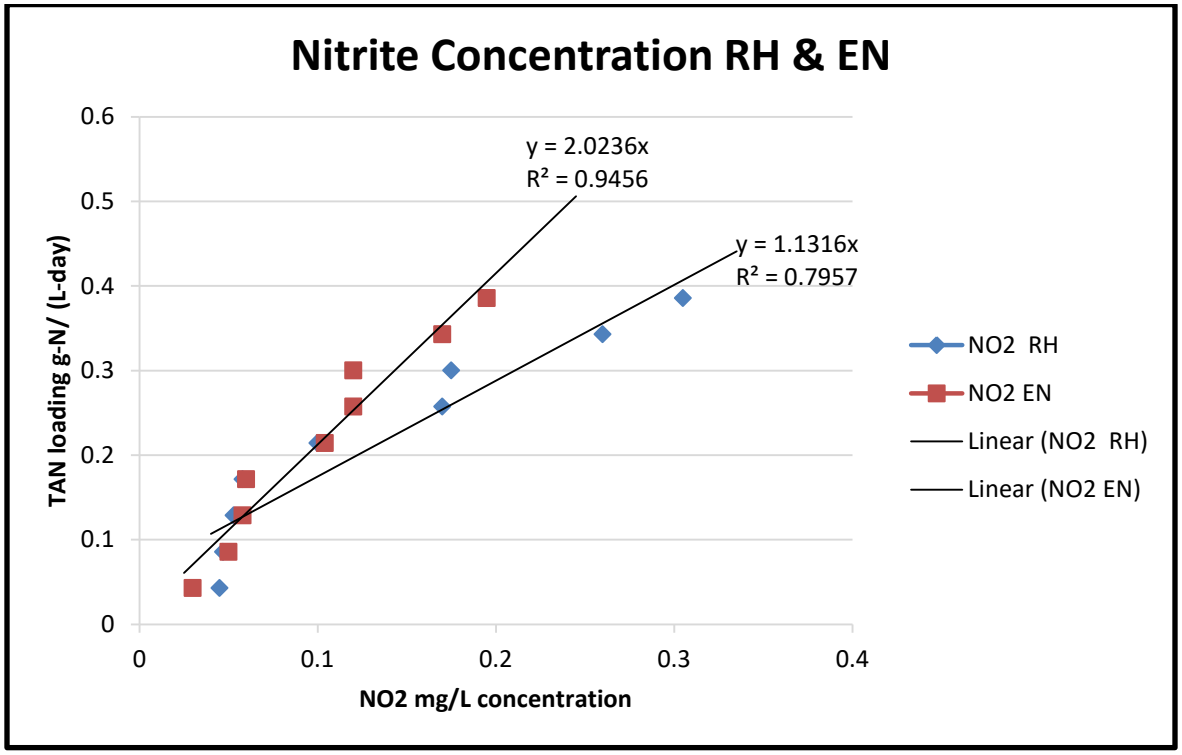

Figure 17: EN and RH media perform at similar levels at lower inflow TAN concentrations. EN bead systems' nitrite concentrations become increasingly lower than RH as the inflow loading increases.

3.5.5. Statistical analysis

A Student t-test was made for ammonia and nitrite concentrations, via SAS software. The Tukey test (α=95) helped determine whether there was a significant difference in VTR based on media type (EN v. RH) and based on loading. Test data were derived from the means of EN

and RH results. Table 4 shows that there is a significant VTR difference between EN and RH media, a significant VTR difference across loading (hence feed rates), and a significant difference for loading*type interaction.

Table 4: Tukey test results demonstrate a significant VTR difference for media type and loading.

Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Media type	1	4	91.94	0.0007
Loading	8	32	46.77	<.0001
Loading*Type	8	32	4.47	0.001

Additional Tukey tests show single-step comparisons between the two media types for NH_3 and NO_2. Results are detailed in Table 5 and Table 6. For both tables, data points with common Tukey letters have no significant difference. Thus, there is no significant difference between RH and EN measurements of ammonia removal until the feed rate reaches 4 g/day/tank, that is, until TAN reaches an oligotrophic level of 0.17 g-N/L/day. There are, however, significant differences between loading levels. Significant difference for nitrite removal starts at the upper oligotrophic level, with heightened contrast at a TAN of 0.3 g-N/L/day and feed rate of 7 g/day/tank. At feed rates between 1 g and 4 g, measurements are similar across loading levels and across media types. At feed rates of 5 g and 6 g, EN and RH systems have similar nitrite measurements. RH nitrite readings at 5 g of feed per day are similar to RH readings at 6 g/day, and similar to EN readings at 6-7 g/day. Likewise, RH readings at 7 g/day are similar to EN readings at 8 g/day; RH readings at 8 g/day are similar to EN readings at 9 g/day.

3.6.Discussion and conclusions

The EN media proved to be an effective floating biocarrier in a 3-phase reactor format. The use of screens was avoided by the gravity clarifier, which in this turbulent regime proved effective at removing dislodged biofilm without loss of beads. High conversion rates were obtained, with a τ average measured at 1219 g-N/m^3-day. The τ of 1.36 g-N/m^3-day from Figure 16 can be used for a 3-phase EN reactor.

The RHBR produced a slope τ of 1.07 g-N/m^3-day in loading conditions of 0.043-0.386 g-N/L/day on figure 16. Results indicated that RH are a viable sinking media for a 3-phase reactor, with a VTR of 1025 mg-N/L/day. RH also surpasses KMT, which has a capacity of 300-400 mg-N/L/day, and the Curler Advance X-1, which has a VTR of 605 g-N/m^3 (Timmons and Ebeling, 2007). RH did biodegrade after 18 days under warm-water conditions.

Despite comparable conversion rates, the two media differ in their life spans. While EN has lifelong durability, RH exhibits decay after 3 weeks. Both systems achieve nitrification performance that can lead to successful growth and production of fish. Results demonstrate that EN beads are more effective at removing TAN, which concurs with findings from previous studies on static bed floating bead filters (Bellelo, 2006), but this experiment differed in that EN is now proved efficient even in an alternative filter (non FBF) context. The RHBR needs an internal clarifier, but the EN beads have clarifying abilities. For this reason, an EN 3-phase reactor will tend to be smaller than a RHBR.

While this study sought to define nitrification performance for the RH reactor, the experiment indicated that the internal clarifier primarily controls the RHBR sizing. This differs greatly from other 3-phase reactors like the MBBR and screens, where media configuration typically controls design. Indeed, these other devices utilize large surface/low SSA media. The

RHBR uses a media with a high SSA, but its small size and sinking properties entail the need for a well-designed, yet limiting, clarifier.

Table 5: Student's t-test results for ammonia

Feed Rate	Obs.	TAN (g-N/L/day)	Type	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1g	1	0.0430	EN	0.0450	0.0048	0.05	0.0353	0.0547	J
	2	0.0430	RH	0.0567	0.0048	0.05	0.0470	0.0664	J
2g	3	0.0860	EN	0.0833	0.0048	0.05	0.0736	0.0931	I
	4	0.0860	RH	0.0907	0.0048	0.05	0.0810	0.1004	HI
3g	5	0.1290	EN	0.1150	0.0048	0.05	0.1053	0.1247	GH
	6	0.1290	RH	0.1223	0.0048	0.05	0.1126	0.1321	G
4g	7	0.1710	EN	0.1100	0.0048	0.05	0.1003	0.1197	GH
	8	0.1710	RH	0.1643	0.0048	0.05	0.1546	0.1741	F
5g	9	0.2140	EN	0.1630	0.0048	0.05	0.1533	0.1727	F
	10	0.2140	RH	0.2000	0.0048	0.05	0.1903	0.2097	E
6g	11	0.2570	EN	0.1583	0.0048	0.05	0.1486	0.1681	F
	12	0.2570	RH	0.2290	0.0048	0.05	0.2193	0.2387	D
7g	13	0.3000	EN	0.2127	0.0048	0.05	0.2030	0.2224	DE
	14	0.3000	RH	0.2657	0.0048	0.05	0.2560	0.2754	C
8g	15	0.3430	EN	0.2283	0.0048	0.05	0.2186	0.2381	D
	16	0.3430	RH	0.3300	0.0048	0.05	0.3203	0.3397	B
9g	17	0.3860	EN	0.3050	0.0048	0.05	0.2953	0.3147	B
	18	0.3860	RH	0.3817	0.0048	0.05	0.3720	0.3914	A

Table 6: Student's t-test results for nitrite

Feed Rate	Obs.	TAN (g-N/L/day)	Type	Estimate	Standard Error	Alpha	Lower	Upper	Letter Group
1g	1	0.043	EN	0.03167	0.008763	0.05	0.01385	0.04949	F
	2	0.043	RH	0.04167	0.008763	0.05	0.02385	0.05949	F
2g	3	0.086	EN	0.04533	0.008763	0.05	0.02751	0.06315	F
	4	0.086	RH	0.04467	0.008763	0.05	0.02685	0.06249	F
3g	5	0.129	EN	0.051	0.008763	0.05	0.03318	0.06882	F
	6	0.129	RH	0.05467	0.008763	0.05	0.03685	0.07249	F
4g	7	0.171	EN	0.05833	0.008763	0.05	0.04051	0.07615	F
	8	0.171	RH	0.06133	0.008763	0.05	0.04351	0.07915	F
5g	9	0.214	EN	0.113	0.008763	0.05	0.09518	0.13082	E
	10	0.214	RH	0.13667	0.008763	0.05	0.11885	0.15449	CDE
6g	11	0.257	EN	0.12	0.008763	0.05	0.10218	0.13782	DE
	12	0.257	RH	0.165	0.008763	0.05	0.14718	0.18282	CD
7g	13	0.300	EN	0.12	0.008763	0.05	0.10218	0.13782	DE
	14	0.300	RH	0.17667	0.008763	0.05	0.15885	0.19449	C
8g	15	0.343	EN	0.165	0.008763	0.05	0.14718	0.18282	C
	16	0.343	RH	0.26333	0.008763	0.05	0.24551	0.28115	B
9g	17	0.386	EN	0.22833	0.008763	0.05	0.21051	0.24615	B
	18	0.386	RH	0.32	0.008763	0.05	0.30218	0.33782	A

As prescribed by Gudipati's (2005) recommendations, an airlift submergence to lift ratio of 3:1 proved efficient in both systems. Based on this ratio, water from the aquarium tank to the reactor has 12 in. submergence and 4 in. lift, with an airflow rate of 1.63 Lpm. The recirculation system utilized gravity to carry the water from the reactor back to the tank. The use of airlifts in both moving bed designs (EN and RH) should be further investigated. Indeed, Malone (2013) mentioned that the constant air injection that expands the media can also "make significant contributions to the RAS aeration and degasification needs." An additional study of the designs presented in this chapter could measure DO, BOD, and CO_2 concentrations and quantify this other beneficial aspect.

According to experimental results, RH proved to be a competitive alternative in ultra-oligotrophic, oligotrophic, and lower mesotrophic conditions. Thus, this chapter recommends RHBR for broodstock, nursery, fingerling, and ornamental applications. Further study should monitor and evaluate the system's nitrification performance for growout, with greater loading.

Malone *et al.* (2006) discussed the complexity of applying Monod kinetics in extreme (high or low) loading conditions. They adapted and developed a model to apply Monod graphs that represent ammonia oxidation in ultra-oligotrophic and oligotrophic RAS. Likewise, future studies should explore the different stages of bacterial growth as well as the log pattern of increase and growth interruption for a RHBR system.

The RHBR remains an attractive option, because it answers the need for green technology referenced throughout the literature on the problem of RAS innovations. RH also answer the cost issue that remains prevalent in developing countries. As EN and other synthetic media remain financially inaccessible in non-western industries, RH can be successfully used as biocarrier with a system τ of 1025 mg-N/L-ppm-day and a design value of 700 mg-N/L-ppm-day

applied to low trophic levels. The experiment also indicates that aquaculturists would need an overall volume of RH much higher than EN, because EN have unending lifetime, whereas RH disintegrate over a wet timeframe. The RH volume required, V_{RH}, to fill and operate the RH filter for "n" weeks is defined as:

$$V_{RH} = V_b + \frac{V_b \times \text{ n weeks}}{3}$$

Nevertheless, the initiative presented in this chapter is worthy to pursue, since cost of new RH for replenishing is virtually negligible. Future research should investigate the effect of temperature on RH system acclimation as well, especially if we consider that developing countries in the global south typically exhibit higher temperatures than the room temperature of this lab experiment.

CHAPTER 4. ENGINEERING OF RICE HULLS AS A BIOCARRIER FOR COMMERCIAL SCALE AQUACULTURE

4.1. Introduction

Contemporary aquaculturists are faced with complex challenges as they must grapple with increased demand for seafood products (White *et al.*, 2004). Indeed, as the population increases, there is a growing demand for healthy fish products worldwide. Multinational corporations' short term and medium-term profits often require endangering and sacrificing ecological balance and social stability. Developments and profits in this industry must be long-term and sustainable. Likewise, Gutierrez-Wing and Malone (2006) addressed a noteworthy concern as they called engineers to research biofiltration technologies that are not only sustainable, but applicable to commonly consumed, large-scale produced species.

Aquaculturists have expressed a need for "greener" commercial aquaculture strategies, and particularly a need to develop more effective, environment-friendly, ways of removing nutrients out of aquaculture water. Recirculating aquaculture systems (RAS) are water-efficient tank water systems that reuse water, minimizing water use. Submerged, fixed-film biological filters are popular for the critical nitrification process. Moving bed reactors are suitable and have high specific surface, as their biocarriers have a shape conducive to maximize bacterial growth. Nevertheless, despite low operational costs, their initial capital costs are considered high in developing countries, partly because of biocarrier costs (Greensword, 2015; Masser *et al.*, 1999). Media substitution with locally available resources can help these countries access moving bed aquaculture.

Fish production is strikingly uneven, leaving areas that, despite having water sources, still lack production to feed their population. Besides Asia, developing countries struggle to either

compete in world market production. Apart from Norway, Chile, Egypt, and Brazil, the top 15 aquaculture producing countries are located in Asia (Wee, 2017). Africa has long coastlines and inland waters, yet its aquaculture production is relatively low and insufficient for the continent's fast growing population.

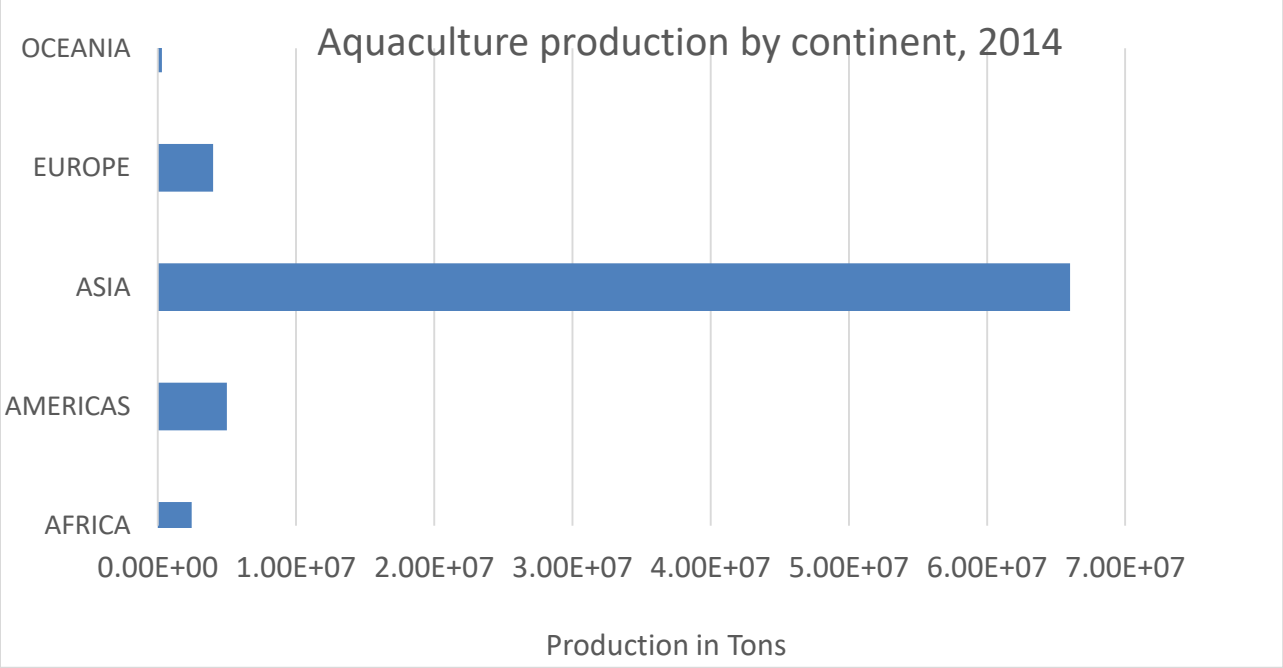

Figure 18: Asian aquaculture dominates world production, and therefore constitutes a favorable context for aquaculture research and innovation.
Source: FAO, 2016

South America has successfully developed production of large tilapia, trout, shrimp, and salmon pond based systems (Watanabe *et al.*, 2002; Garcia *et al.*, 2013). Yet, these successes have been overshadowed by environmental impact and disease problems. African and Latin American aquaculture industries are yet to partake in the rise in RAS use. Bobstock *et al.* (2010) observed that these countries lack the information systems and scientific support and research

sectors required to support expansion. Africa and South America's absence from the United Nation's Food and Agriculture Organization (FAO) database of regional capture statistical collections (FAO, 2017) illustrates how excluded these developing areas are from the fishery production global scene. Investors are few and domestic institutional support remains insufficient. The major viable option for such industries is self-reliance on local infrastructures using domestically available resources.

4.2. Objectives

This study utilizes previously established data to scale a RH reactor to a commercial fingerling production facility. The objectives are to produce a commercial-scale design that can accommodate the production of 52,900 kg fingerling/year. Then, a cost analysis aims at determining whether RH-assisted aquaculture is beneficial to developing countries, as opposed to other media configurations, namely EN and KMT. To do so, the economics section of this chapter seeks to produce a capital cost analysis based on reactor built, and an operation cost analysis, based on media management.

4.3. Background

4.3.1. Cost of biofiltration

Greensword (2015) completed a cost analysis of a large-scale tilapia production facility using an airlifted-PolyGeyser® RAS in the U.S. The hypothetical facility contained a fingerling and a growout section. Calculations showed that capital costs to produce 52,900 kg (116500 lbs.) fingerlings amounted to $493,700, among which $441,700 was allocated to RAS equipment alone. The equipment production cost for the entire growout facility is $0.26 per pound of fish.

The study of Westerman *et al.* (1993) determined the cost of a fluidized sand filter to be $1000 for a lifetime of 10 years. Loading calculations showed that the filter could remove 51 g TAN/$.

Losordo and Westerman (1994) conducted a cost analysis of a small commercial RAS via computer simulation to determine the costs of producing 43,500 kg of tilapia per year. The nursery biofiltration system was a combination of one floating bead filter (FBF) (lifetime 10 years) and one RBG (lifetime 7.5 years) attached to three tanks. The reactors cost $8,000. Based on reactor volumes, TAN v. cost calculations indicated that the RBC removed 53 g TAN/$, and the FBF 20 g TAN/$. Nevertheless, the FBF was installed to both complement RBC biofiltration and to remove solids.

Adler *et al.* (2000) conducted a costs analysis and concluded that high ownership and operation costs are the result of aquacultural wastewater treatment whether with chemical precipitation, physical removal, or land application technologies. Their study detailed that a lettuce aquaponics with a fluidized sand system with 20 years' lifetime allowed them to produce 22,680 kg trout from hatchery stage to growout. The $32,300 system also contained recirculation pumps, CO_2 stripper, oxygen equipment, and a drum filter.

4.3.2. Affordability of aquaculture in developing countries: the example of tilapia in India and Côte d'Ivoire

Over two decades ago, Davlin (1991) called for aquaculture engineering to help counter the insistent poverty of producers in developing countries: "the technological breakthroughs of academia/marine biologists are needed to transform aquaculture into a source of income in developing country farmers." Today, India demonstrates great efforts to implement RAS technologies into its aquaculture industry (Kumar *et al.*, 2009), as commercial aquaculture is

considered a strategy to aggressively counter the country's poverty and malnutrition crisis that affects its rural areas (Azim *et al.*, 2005). Nevertheless, critics argued that modern aquaculture is not tailored to developing countries (Jana and Jana, 2003). While mangrove swamps have shown to be adequate for commercial hatcheries and nurseries, they are only fit for marine production, which only accounts for 23% of Indian catch fish production, and less than 5% of the national fish production (Anneboina and Kumar, 2017; Mohan and Bhatta, 2002). India's largest production, freshwater aquaculture, is mainly conducted as pond and cage aquaculture (Rao *et al.*, 2013). However, these techniques have been correlated with fish disease and biofouling problems due to uncontrolled bacterial, parasitic, and fungal activity (Mohan and Bhatta, 2002; Rao *et al.*, 2013; Bhaumik *et al.*, 1991). These problems can be countered by the use of chemicals, but this also generates environmental concerns (Pathak *et al.*, 2000). India is also making efforts to diversify its cultured species. While carp is its number one production, tilapia is becoming an attractive option, because it is a hardy species, adaptable, and tolerant to different nitrite water conditions (Malone and Pfeiffer, 2006; Chakraborty *et al.*, 2010), although it was rejected previously (Pullin, 1996).

Tilapia is originally an African species, but African countries have encountered numerous difficulties culturing it. Côte d'Ivoire is among its largest producer in Sub-Saharan Africa (FAO, 2016). Ivorian tilapia is mainly grown as lagoon, pens, acadjas (where branches are used as artificial reef where natural fish feed is grown) and pond culture (Pullin, 1996; Chikafumbwa, 1996; Rose *et al.*, 2017; Bamba *et al.*, 2014; Blay, 2015; Durand and Hem, 1996). Pullin (1996) detailed the problems and needs of tilapia farming systems in Côte d'Ivoire in the 1990's, as detailed in Table 7.

Table 7: Problems associated with tilapia farming systems (Pullin, 1996, p. 14)

System	Major problems	Farmers' needs
Cages	*Ad hoc* design, guessed at or copied from elsewhere; poor feed conversion; fouling; short operational life.	Systems specifically designed for tilapias in fresh-, brackish- and saltwater.
Pens. acadja-enclos, etc.	Still experimental.	Reliable, sustainable systems that match their resources.
Ponds	Nutrient starvation; *ad hoc* stock management; water availability/quality.	Sustainable systems, well-integrated with other enterprises.
Tanks, raceways and other intensive systems, including recycling	Largely experimental or guesswork at site-specific designs.	Reliable guidelines-as exist for trout culture.
Hatchery/nursery systems	Low and/or seasonal output of fry/fingerlings; no consideration of genetic consequences of broodstock management; low adoption of monosex seed technology.	Reliable seed supply systems that maintain genetic quality and 100% male seed production, where such is appropriate.

Nowadays, these problems still persist, mostly due to insufficient financial and biotechnological investment. In addition, pollution has severely worsened lagoon conditions (Scheren *et al.*, 2004; Coulibaly *et al.*, 2012; Cyrille *et al.*, 2012).

Whether in India or Côte d'Ivoire, small-scale farmers remain the backbone of aquaculture in developing countries, which limits their marketing abilities. Expanding sustainable production would require technology financially available to rural farmers in terms of

costs, ease of construction, and informed management plan (Kutty, 2005; Bhatta and Bhat, 1998; Lazard and Weigel, 1996).

4.3.3. Rice production and rice hulls

The pairing of fish and rice culture has already been explored in terms of culture-site rotation for human consumption, in China and Bangladesh notably (Weimin, 2010; Kutty and Weimin, 2010; Xie *et al.*, 2011; Ahmed and Garnett, 2011; Ahmed and Granett, 2010; Dey *et al.*, 2013; Ahmed *et al.*, 2011). Developing countries with coastal and inland waters commonly have high rice production, as shown on Figure 19. Côte d'Ivoire and India have also developed models for fish feed using rice bran and other rice refuse (Morissens *et al.*, 1996; Dharmaraj and Dhevendaran, 2010) or other locally grown resources (Costa-Fierce, 1996).

Rice is abundant in Côte d'Ivoire, a developing country that produces enough of the crop to feed its population and export globally, to the extent that the nation hosts the African Rice Center and bears the nickname of "West Africa's Rice Bowl" (Assi *et al.*, 2013; Abe *et al.*, 2010; Seck *et al.*, 2012; Edi *et al.*, 2012; GrowAfrica Secretariat, 2017). Yet, the availability of rice as a co-product or feed has not remedied the developing world's insufficient fish production, as shown on Figure 19. Little literature is available regarding the local or national access to Indian RH. In Côte d'Ivoire, RH are considered waste, and are commonly burnt after grain extraction, creating a pollution problem.

In the case of India, which is a significant producer of both fish and rice, seizing the momentum of RAS innovations could help this developing nation reach its goal to satisfy its growing domestic fish demand and its 15 million tons production goal for 2020 (Anon, 1999; Press Information Bureau, 2016). Using an abundant resource such as RH to enhance production also seems to be a lucrative option, especially given the recent interest in the sale of Indian RH

(Gidde and Jivani, 2007; Mohan, 2013). Current RH prices average at $0.04/kg. While importers would purchase RH at $30/ton in 2013, prices can now go up to $77/ton, based on rice type in 2017 in India. India exports most of its RH to Europe.

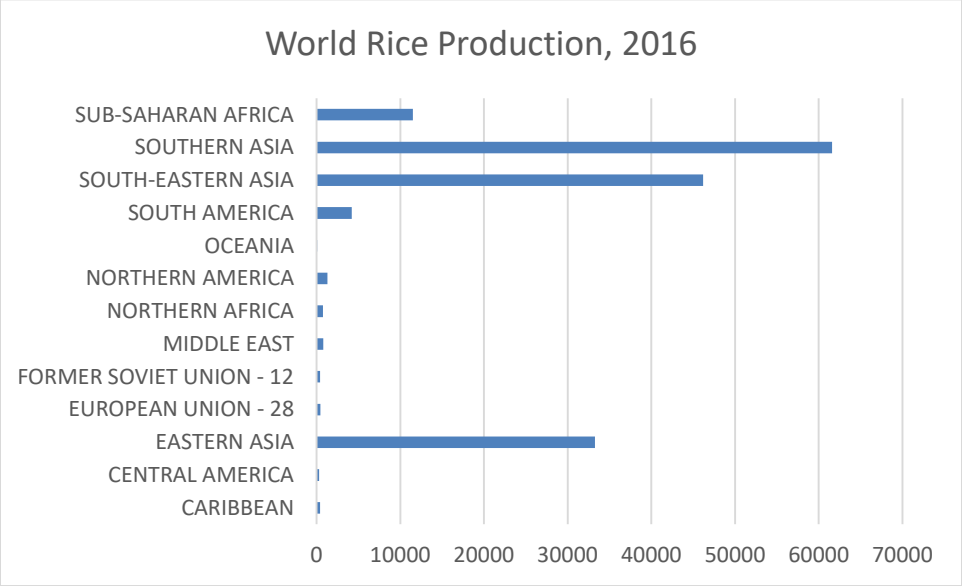

Figure 19: Sub-Saharan Africa, South America, and proportionally the Caribbean play a considerable part in the global rice production, which contrasts with their relative invisibility in the global seafood production.
(Source: International Rice Research Institute, 2017)

4.3.4. Biofiltration and biofilter design for facilities

Biological filters are a necessary part and dynamic variable in the design of recirculating aquaculture facility units. Filters determine the size of the facility and the cost of its ownership and operation (Ernst *et al.*, 2000). Because RAS facilities are particularly vulnerable to nitrite poisoning, they must be designed with a carefully established fish to feed ratio and proceed to regular water quality control and administer proper management of biological filters (Svoboda et al, 2015). For example, Terjesen *et al.* (2008) provide dimensions for a bioreactor-equipped

research facility in Norway with experimental and growout halls. Engineering based on loading drove all major costs, and the size of equipment was dimensioned based on this parameter.

4.4. Adjusted design

Figure 20 is a 2D schematic of the RHBR-equipped RAS. Although experimentally, the design addressed sludge removal and solids capture via filter pad, it is recommended to supplement this commercial-scale biofilter with another low-cost clarification device. Thus, the entire RAS consists of four 835 liter tanks length 24.5m, width 12m and depth of 0.75m and, each connected to a RHBR on one end and a clarifier on the other for solids capture. Airlifts and airstones in the tank address the need for aeration, circulation, and degassing. The RHBR provides biofiltration, and the clarifier removes solids.

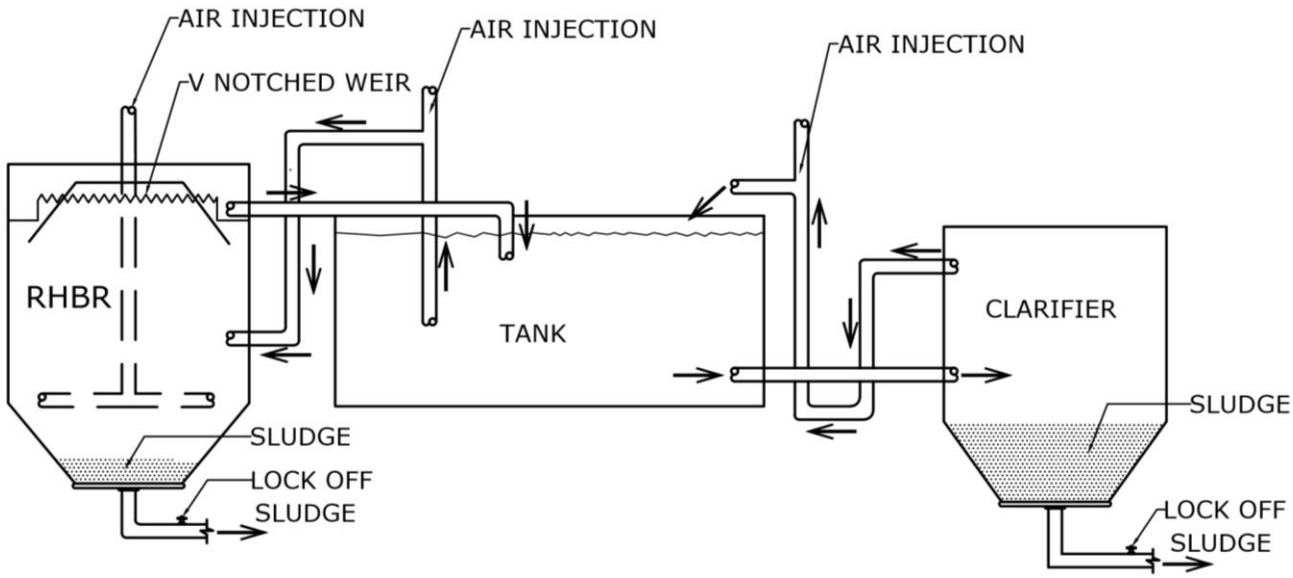

Figure 20: The adjusted RAS contains airlifts and airstones for aeration, degassing, and circulation, a RHBR for biofiltration, and a separate clarifier.

4.4.1. Filter design criteria

With a design set for low loading aquaculture, the proposed hypothetical facility is considered for tilapia fingerling production. Since smaller farmers are the backbone of aquaculture production in developing countries, the production capacity of the hypothetical facility was set at 52,863 kg/year harvest at 70 g (after an assumed 10% stocking fingerling loss), which constitutes an amount appropriate for commercial scale. To that end, 12,082 kg fingerlings at 16 g each are placed into 4 tanks, each connected to a RHBR. As per the cost analysis previously conducted by Greensword (2015), they remain in the fingerling tanks for a cycle of 21 weeks, at the end of which they have grown 70 g in weight and above 2.5 cm body length (Bocek, 2009; Abernathy, 2015). Upon completion of a cycle, the fish are transferred to a growout facility. Operation is assumed at 2.5 cycles per year. The corresponding daily feed for fingerlings is 6 kg/tank-day amounting to an annual feed of 79294.5 kg/year for the facility. Chapter 3 showed that RH can operate at a VTR of 1025 mg-N/L/day. For a commercial-scale facility, a more conservative design value of 700 mg-N/L/day was adopted. The corresponding amount of media needed is 0.32 m^3 (wet volume) of husks per tank (that is, 1.28 m^3 for the facility). Media must be replenished in full every 3 weeks, leading to 7 replacements per cycle, and 17 replacements per year. The facility needs 44 m^3 RH per year for its RHBR (11 m^3/tank). Values are summarized in Table 8.

4.4.2. RHBR design

Figure 21 provides a 3-D view of RHBR designed to host 181 m^3 of RH. With a hull ratio of 4:1 and 3 m diameter, the bioreactor has a volume of 45.3 m^3. The total container is 2 m high and has a volume of 8.5 m^3. The tank water enters the filter at Q_{in} (1), discharging into the

reactor zone (2). An air injection tube inserted in zone (3) branches into 2 airstones (4) and (5) inside the conic reactor (2) where the media bed is expanded.

Table 8: RHBR sizing parameters for an 52,863 kg/year tilapia fingerling production facility

Item	Amount	Unit
Annual harvest	52,863	kg
Peak daily feed rate	1057.26	kg feed
Peak nitrogen excretion	31717.84	g-N/day
Media amount	45.3	m^3
Container Volume	181	m^3

After 18 days in the reactor, RH take 3 days to fully deplete, during which they are progressively captured into the chamber (7). It is assumed that 1/3 media volume depletes daily for 3 days, leading to a sludge production of 0.19 m^3/day for 3 days every 21 days. An internal sludge chamber or pipe (7) captures depleted media. Sludge is thus evacuated via the sludge removal pipe (7). Inversely, 0.19 m^3/day are added every day for 3 days, every 3 weeks. The weir in zone (6) collects the treated water that is then returned to the tank through Q_{RH} (8). The flow rate Q_{RH} is determined by assuming 100% removal rate and neglecting the *in situ* nitrification. The calculated loading from Table 8 of 31,718 g-N is represented by the following equation:

$$\text{Loading} = Q_{RH} * TAN_{C,}$$

where TAN_c represents the TAN critical constant at 2 g/m^3-day. Therefore:

$$Q_{RH} = \frac{\text{Loading}}{TAN_C} = \frac{31{,}718 \text{ g-N}}{2 \text{ g/m}^3\text{-day}} = (15{,}899 \text{ m}^3/\text{day}) \left(\frac{1000 \text{ L}}{\text{m}^3}\right) \left(\frac{\text{day}}{1440 \text{ min}}\right)$$

$$Q_R = 11{,}013 \frac{\text{L}}{\text{min}} \Rightarrow \frac{2{,}909 \text{ gpm}}{\text{system}}$$

With a system flow rate of 2,909 gpm, each tank has a flow rate of 727gpm.

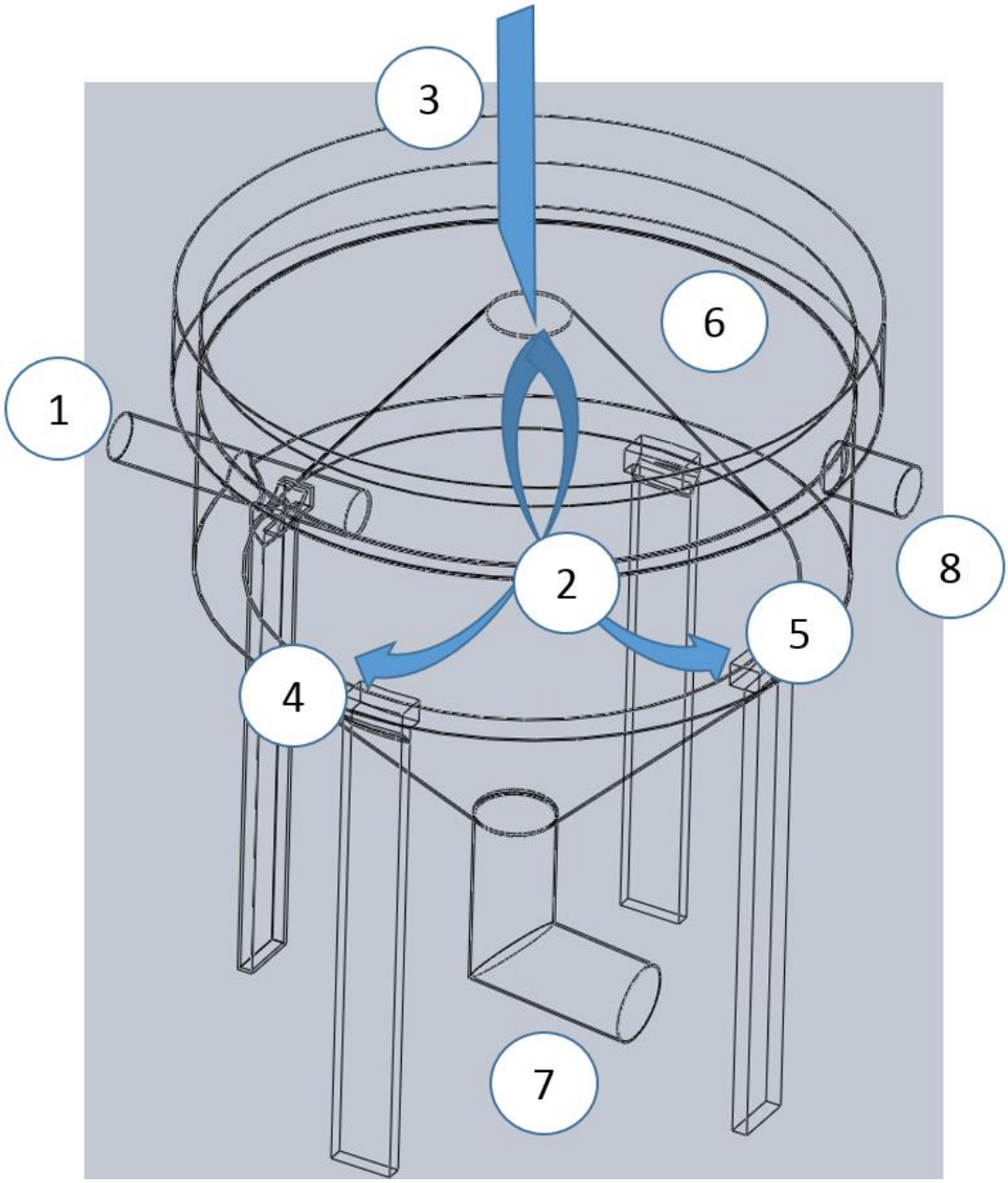

Figure 21: The commercial-scale RHBR contains an internal clarifier to prevent rice hull loss while removing sludge created.

4.4.3. Clarifier design and model

The circular clarifier design was sized based on feed F and excretion rate E_{TSS}. A mass-balance approach (Figure 22) for all 4 tanks helped determine the amounts of total suspended solids (TSS) in the tank to be removed by the clarifier, hence its size, its radius r, and area A. TSS is defined as the product of feed (2331. lbs. or 1057.26 kg per feed per day), and excretion rate E_{TSS} (0.5 kg-TSS/kg-feed). A 100 micron particle size is assumed (Metcalf and Eddy, 2014). C_T is the TSS concentration in the solids to be removed from the tank, assumed at C_T = 200 g/m^3. V_0 is the standard clarifier's overflow velocity, set at 934 m^3/day/m^2 (650 gpd/ft^2).

$$FE_{TSS} = (1057\ \text{kg/day})(0.5) = \frac{528.63\ \text{kg}}{\text{day}}$$

$$\frac{dTSSt}{dt} = F \times E_{TSS} - Q_R^C C_T$$

At steady state,

$$Q_R^C = \frac{F \times E_{TSS}}{C_T} = \frac{528630\ \text{g/day}}{200\ \text{g/m}^3} = 2640\text{m}^3/\text{day}$$

$$Q_R^C = \left(\frac{2640\ \text{m}^3}{\text{day}}\right) \times \left(\frac{1\ \text{day}}{1440\ \text{min}}\right) \times \left(\frac{264\ \text{gal}}{\text{m}^3}\right) = \frac{484\ \text{gal}}{\text{min}}/4\ \text{tanks} = \frac{121\ \text{gal}}{\text{min}}/\text{tank} = \frac{0.46\ \text{m}^3}{\text{min}}/\text{tank}$$

The clarifier's diameter is determined by the overflow rate (V_0) of 26.5 m^3/m^2-day (650 gpd/ft^2) and the flow rate (Q_R).

$$V_0 = \frac{Q_R^C}{A} \Rightarrow A = \frac{Q_R^C}{V_0}$$

$$A = \frac{121 \times 1440}{\frac{650\ \text{gpd}}{\text{ft}^2}} = 268\ \text{ft}^2$$

$$A = \pi r^2 \Rightarrow r = \left(\frac{A}{\pi}\right)^{1/2} = \left(\frac{268.06\ ft^2}{3.14}\right)^{1/2} = 9.24 ft. \times \left(\frac{0.3048\ m}{1\ ft.}\right) = 2.82\ m$$

$$\text{HRT} = \frac{8.5\ \text{m}^3}{2640\ \text{m}^3/\text{day}} \times \frac{1440\ \text{min.}}{1\ \text{day}} \approx 5\ \text{min.}$$

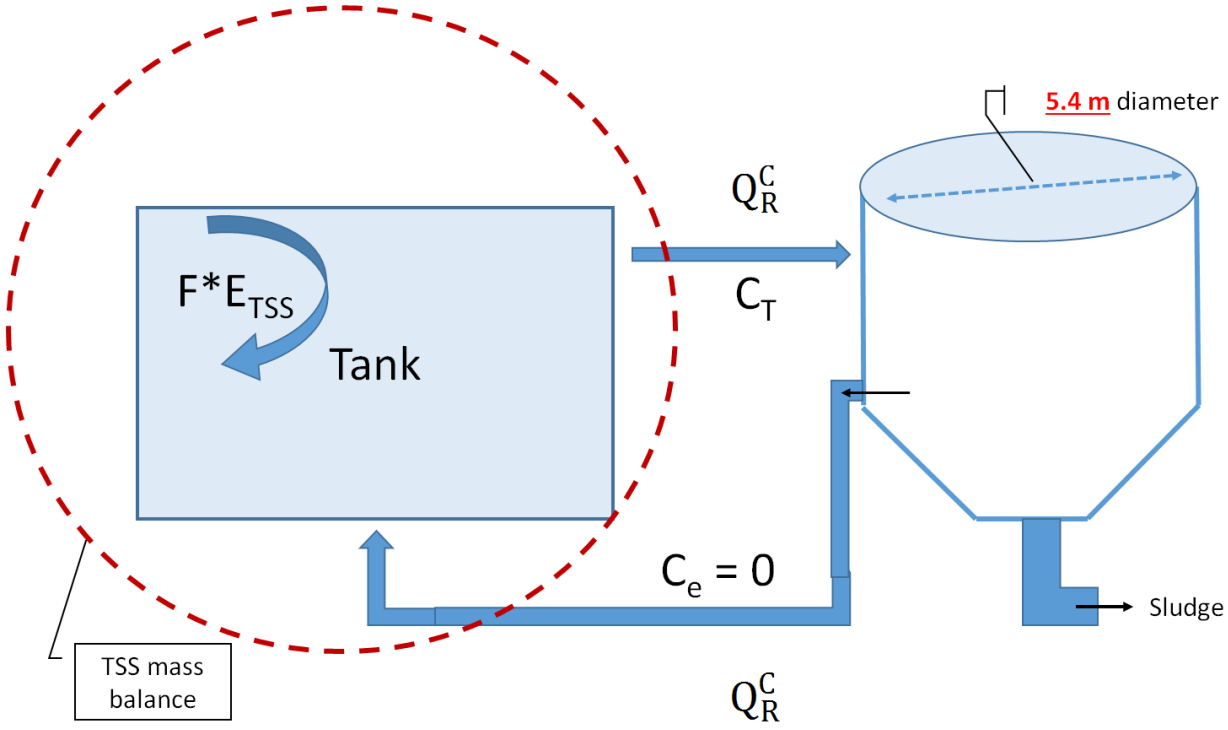

Figure 22: Mass balance calculations for the clarifier of an RHBR-equipped fingerling production facility lead to a clarifier diameter of 3 m.

The clarifier has a volume of 40 m^3 and an overall height of 2 m. As the water enters at Q_{R1}, it remains in the clarifier for a retention time of 5 minutes, based on Q_R^C that allows for sedimentation. The solids settle at the bottom and are evacuated through the sludge chamber. Clarified water is then returned to the tank at Q_R^C.

4.5. Cost analysis

Ownership refers to the annualized costs associated with initial and replacement equipment, equipment operation, and maintenance. Ownership costs for a RHBR were determined, first using U.S. prices. After a survey of current market prices, these values were found to be comparable to other western areas such as the E.U. and Canada. Costs were then substituted in the template with prices for India and Côte d'Ivoire[6], based on the current literature and market data available.[7]

Based on the materials used (mainly fiberglass and PVC), the filter's useful life is determined to be 20 years. Table 9 details capital costs using current US prices for an entire facility. RHBR and clarifier costs are determined by current fiberglass price. Electricity costs as based on operation requirements for a 100 cfm Pentair pump HPB200 model. Net present value (NPV) was used to determine the 20 year costs for equipment that need replacement, due to a useful life of less than 20 years. These include pipes, airstones, blowers, pipe fittings, RH, electricity, and maintenance. While RH sell at $40/ton in the U.S., transportation (hauling labor and vehicle rental) costs amount to $300. Nevertheless, areas like Louisiana have a well-developed rice and RH production, which means costs could be less depending on the U.S. location. Inversely, Europe and Canada import most of their RH, which would lead to higher transportation costs.

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[6] A survey of market prices in India and Côte d'Ivoire revealed that RHBR materials and resources such as PVC, fiberglass, and labor are similar in both countries. The major difference in prices resides in the fact that RH have marketable value in India, while in Côte d'Ivoire they are treated as waste. Thus, the cost analysis was completed in the assumption that Ivorian RH could be sold at the price of Indian RH.

[7] For comparative purposes, costs were also adapted to a RAS facility equipped with an EN 3-phase reactor (EN3-P) that contains an internal solids capture and sludge chamber. A design of this alternative EN3-P commercial design is presented in APPENDIX D.

Total ownership costs amount to $0.11/lb. fingerling for fingerlings produced over a 20 year period. (Table 9) a comparative analysis showed that using an alternative 3-phase reactor with EN yields ownership costs of $0.05 (see APPENDIX D). The major drivers of RHBR costs were associated with storage space and maintenance, when compared to the EN reactor.

Table 10 is a similar synthesis of costs, with values adjusted to prices in India and Côte d'Ivoire markets, as these locations have similar cost structures. Local, non-imported RH are estimated to be 40% less expensive than in the U.S. A survey of market prices was completed and helped determine that fiberglass costs in India and Côte d'Ivoire are comparable to prices in the U.S., Canada, and Europe prices, and were therefore assumed to be the same. Nevertheless, current GDP's and the local costs of life make fiberglass proportionally more expensive in the developing world. The 100 cfm pumps available locally with similar design and lifetime were on average cheaper than the Pentair models utilized in more developed countries.

While initial equipment generated a system cost of $7,340, media replacement and its associated expenses generated an increase of over $40,000, to reach $0.06/lb. fingerling produced. This cost remains attractive, when considering the high price of other media, such as EN. In addition, the unavailability of EN beads, and the subsequent need to import, would generate transport costs that would be minimal if using local RH, despite the RHBR RAS' storage and external clarification requirements. The total cost of western, RHBR facility ownership approximates $101,600 and ownership in a developing country approximates 47,700. Western aquaculturists can expect a budget that is 253% that of Indian or Ivorian facility owners.

A distribution of costs associated with commercial RHBR fingerling production is presented in Figure 23 and Figure 24. In both economies, the RHBR itself accounts for 1% of

total ownership. The external clarifier (which would not be needed if using clarifying EN biocarriers) also accounts for 1% of costs. Operation and maintenance are the major drivers.

Table 9: Ownership costs for an RHBR using U.S. market prices amount to $0.11/lb. tilapia fingerling over 20 years.

Ownership Cost for a RHBR Fingerling Facility in the West					
Parameters	**Sizing**	**Units**	**Unit Cost**	**Facility**	**Useful Life (years)**
Equipment					
RHBR	45.31	m^3	$26.18	$1,186.25	20
Piping PVC and fitting	500	in.	$0.10	$200.00	20
Air stone	150		$5.00	$750.00	10
Air blowers	100	cfm	$10.08	$1,008.00	7
Construction installation	10	hrs	$25.00	$1,000.00	20
Piping fitting	30	hrs/tank	$20.00	$2,400.00	20
Storage room	625	ft^2	$15.00	$9,375.00	20
Total equipment cost				$15,919.25	
Operation and maintenance					
Rice hulls cost	4	ton	$340.00	$11,578.50	20
Electric cost	8760	kwh	$0.18	$13,424.24	20
Maintenance and replacement cost	150	hrs	$15.00	$19,155.60	20
Risk	500	yr		$4,256.80	20
Labor for refill	365	hrs/yr	$12.00	$37,289.57	20
Total labor cost				$85,704.71	20
Total cost				$101,623.96	
Number of years	20				
Interest rate	10%				
Investment interest rate	3%				
Fingerlings amount	116,298.75	lbs.			
Annuity cost	$11,936.71				
Ownership cost per lb. of fingerlings	$0.11				
Cost per lb. of feed	$0.07				

Table 10: Ownership costs for an RHBR in a RAS facility using India and Côte d'Ivoire prices amount to $0.06/lb. tilapia fingerling over 20 years.

Ownership Cost for a RHBR Fingerling Facility in a Developing Country					
Parameters	**Sizing**	**Units**	**Unit Cost**	**Facility**	**Useful Life (years)**
Equipment					
RHBR	45.31	m^3	$18.70	$847.32	20
Piping PVC and fitting	500	inches	$0.10	$200.00	20
Air stone	150		$5.00	$750.00	10
Air blowers	100	cfm	$8.35	$835.00	7
Construction installation	10	hrs	$6.00	$240.00	20
Piping fitting	30	hrs/tank	$6.00	$720.00	20
Storage room	625	ft^2	$6.00	$3,750.00	20
Total equipment cost				$7,342.32	
Operation and maintenance					
Rice Hulls cost	4	ton	$150.00	$5,108.16	20
Electric cost	8760	kwh	$0.08	$5,966.33	20
Maintenance and replacement cost	150	hrs	$6.00	$7,662.24	20
Risk	350	yrs.		$2,979.76	20
Labor for refill	365	hrs/yr	$6.00	$18,644.78	20
Total labor cost				$40,361.27	
Total cost				$47,703.59	
Number of years	20				
Interest rate	10%				
Investment interest rate	3%				
Fingerling amount	116,298.75	lbs.			
Annuity cost	$5,603.25				
Ownership cost per lb. of fingerlings	$0.06				
Cost per lb. of feed	$0.04				

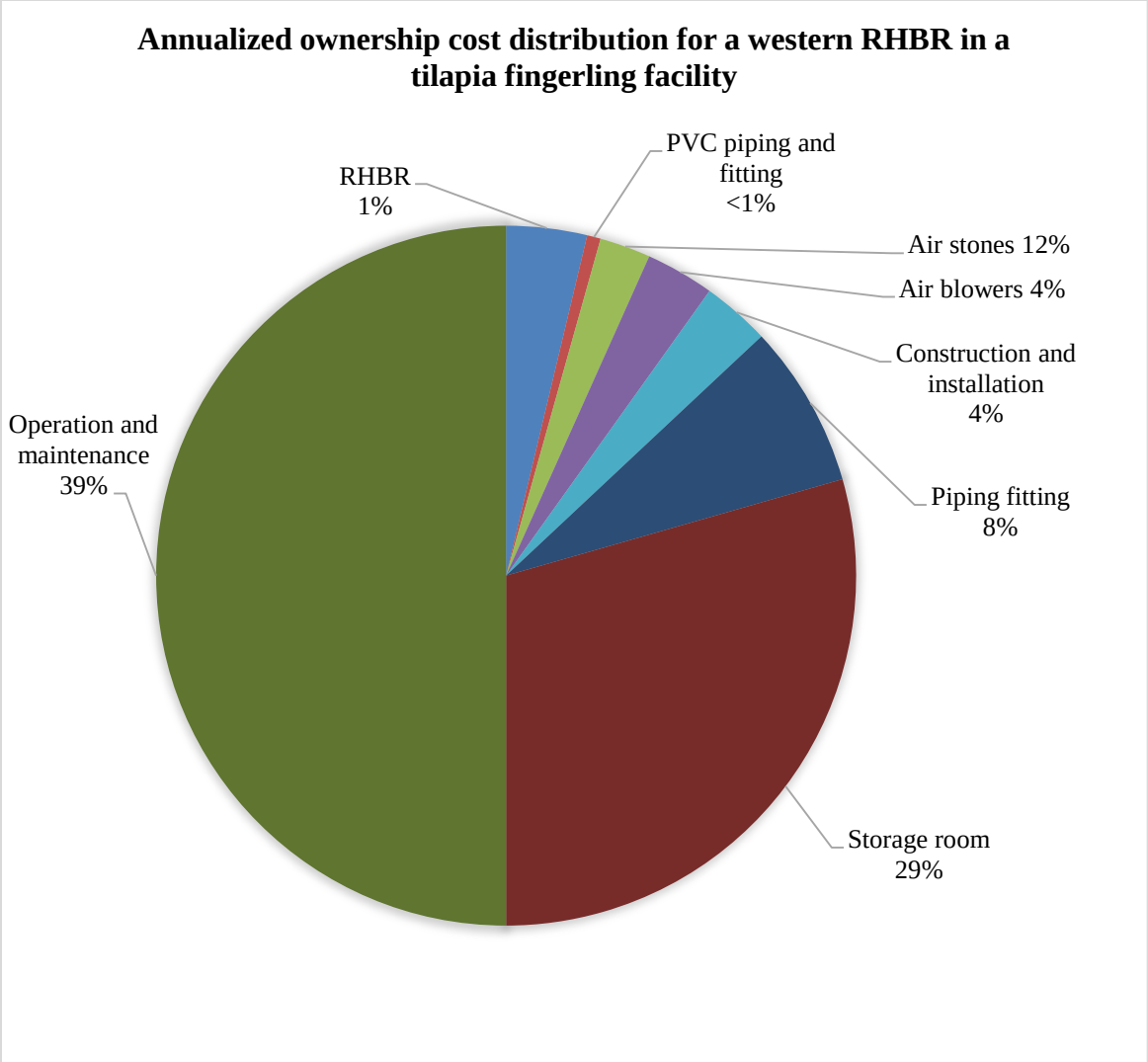

Figure 23: For western facilities, operation and maintenance account for the majority of costs, followed by storage costs associated with RH media refill requirements.

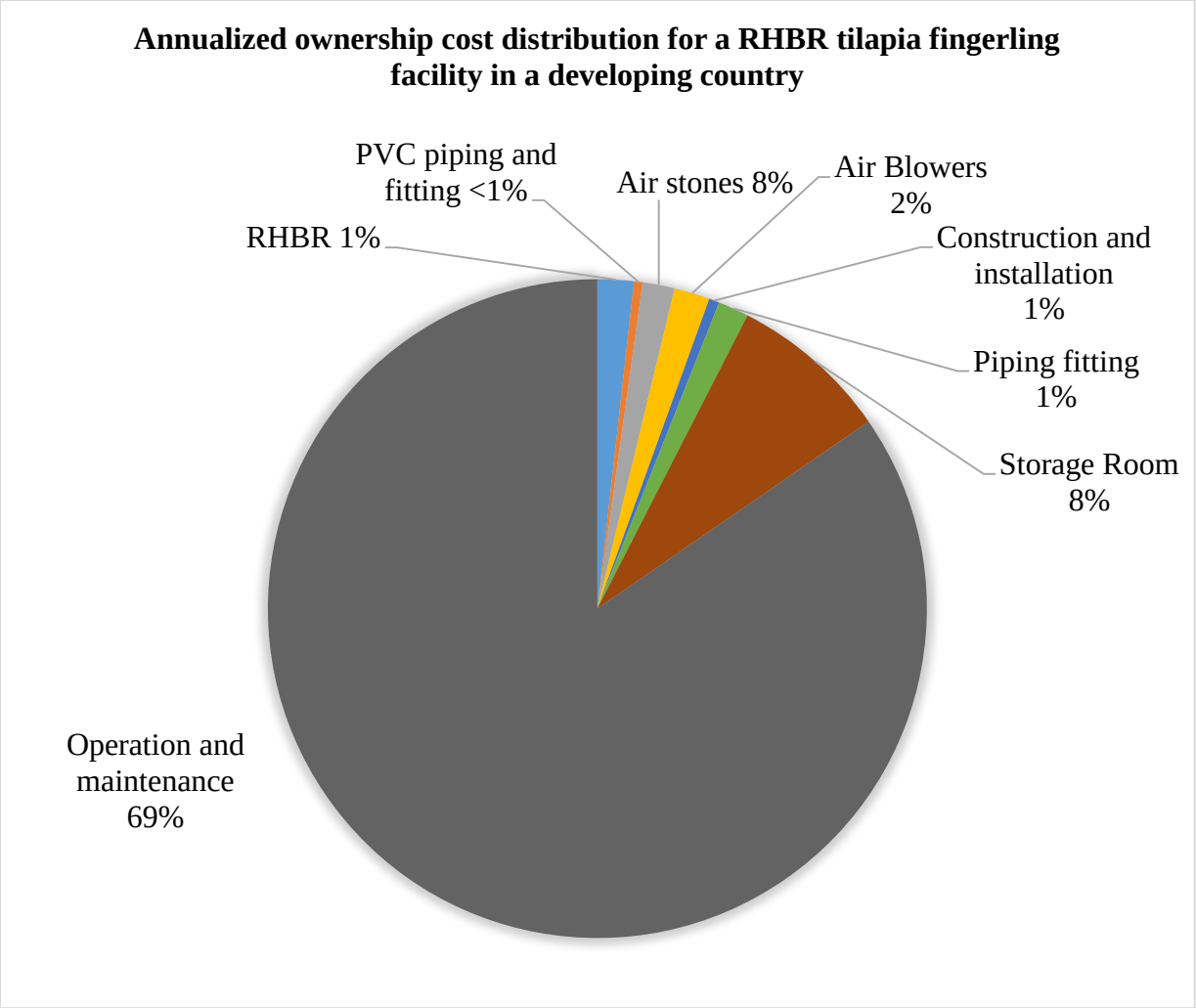

Figure 24: In developing countries, operation and maintenance account for over 2/3 of costs. Storage costs associated with RH media refill requirements are proportionally lower than western facilities.

4.6. Discussion and conclusions

For low trophic commercial-scale applications, a RHBR design should be sized at media VTR 700 g-N/m^3-day. Assuming a feed rate of 260 kg/tank/day and a subsequent nitrogen loading of 7930 g/day, the filter needs 0.32 m^3 RH media. The RHBR's need for an internal clarifier, however, controls a significant part of the design. Indeed, the high SSA RH media is too small to screen. Thus, the internal clarifier prevents media loss and clogging in the RHBR.

Furthermore, the media depletion every 3 weeks creates sludge inside the reactor, and thereby hinders TAN removal if this newly created sludge is recirculated into the tank. Consequently, media replacement must occur 17 times yearly, leading to a RH need of 11 m^3/tank/year.

The RHBR design is also limited due to the need for clarification of suspended solids (fish excrements and feed waste), whereas other systems such as the PolyGeyser® have the advantage of operating with media that nitrify and clarify simultaneously. Contrastingly, an external clarifier is designed to treat an estimated solids amount of 52,863 g/day/tank (0.5 g solids/g feed).

Media replacement and replacement-related expenses constitute a cost of $67,000 and $31,000 in the U.S. and in developing countries, respectively over a 20-year period. This leads to a total lifetime ownership cost of $136,900 in the U.S., and $38,546 in a developing country. The major cost drivers are operation, maintenance, and storage space. The external clarifier is a critical component because it determines the size of the entire facility. While the media is inexpensive, storage space – which also determines the facility's size – constitutes an adjustment that other life-long plastic media do not require.

The RHBR system is overall less expensive than a KMT system, because of the cost of KMT media. For developing countries like India or Côte d'Ivoire, this cost would be heightened because of KMT transportation costs, since this media is typically manufactured in Europe and North America. Likewise, EN beads are more easily manufactured in more developed countries. On the other hand, RH cost and its availability as a media in developing countries make RHBR a viable, attractive option for rice-producing countries.

In addition, several measures could be taken in practice to lower cost presented in this hypothetical cost analysis. First, using concrete instead of fiberglass could reduce equipment cost

by at least 25%, based on current market prices in India and Ivory Coast, although concrete is more tedious to clean and is subject to eroding after a period of time. Then, the virtually constant availability of rice husks in these regions, and the facility's proximity to a mill, could significantly reduce – or even eliminate – storage costs. Indeed, the concurrent culture of rice and fish has gained great interest among Indian and Ivorian agriculture industries (Morissens *et al.*, 1996; Dharmaraj and Dhevendaran, 2010). Finally, labor was generously estimated at $10/man-hour in developing countries. In practice, it can be assumed that, based on local cost of living and per capita income data for India and Côte d'Ivoire, these costs could be drastically reduced.

EN media proved to be economically beneficial in western countries like the U.S. Effectively, an RHBR's ownership costs amount to 212% those of a facility using a 3-phase EN reactor. This facility would, however, be 168% more expensive to own than a RHBR facility in a developing country. Media costs are the major cost drivers for EN aquaculture in the developing world. Furthermore, having to import media would counter these countries' effort to promote self-reliance.

It is recommended that a RHBR prototype be built and tested in an aquaculture facility for fingerling and growout production, in a commercial setting. Water samples should be collected to measure TAN removal, TSS, BOD, and DO. The cost analysis should also be supplemented by practice data. Furthermore, the RHBR that functioned as an activated system with high SSA media conducive to bioactivity created more sludge from decayed media. Elaborating a Monod curve would help quantify the bacterial conversion rate (Malone *et al.*, 2006).

CHAPTER 5. SUMMARY

This dissertation documents the design of rice hull bioreactors (RHBR) for low trophic level aquaculture such as broodstock, hatcheries, fingerling, and ornamental applications. It also sets guidelines for the operation of 3-phase reactors and their internal clarifiers. The filter's volume V_R is determined by the application's loading, knowing that the RH media display a VTR of 1025 mg-N/L/day, adjusted to 700 mg-N/L/day for a commercial scale RHBR, with τ at 1.07 g-N/m^3/ppm, in ultra-oligotrophic to lower mesotrophic waters. The hull ratio of 4:1 sets the biofilter size as:

$$V_R = \left(\frac{\text{Loading}}{\text{VTR}}\right) \times 4 = \left(\frac{\text{Loading}}{700\ \text{mg} - \text{N/L/day}}\right) \times 4$$

A submergence to lift ratio of 25 percent is recommended for PVC airlifts in the RHBR design. However, water and air flow rates should be adjusted to fit the tank's size. Thus, while biofiltration is verified, the design of a larger-scale RHBR is controlled by the need for clarification.

Although RH have a superior SSA and demonstrated good nitrification comparable to other well-acknowledged, life-long media like KMT and EN beads, they lacked these media's durability and clarifying capacity. Thus, an effective RAS with a 3-phase RHBR should include an internal clarifying weir to manage biodegrading media and a separate clarifier for capture of solids generated in the tank or biosolids escaping the RHBR. Chapter 3 shows that for the same media volume, RH nitrification rate is comparable to EN. Nevertheless, RH, as a biodegradable biocarrier, need to be partially replaced every 3 weeks. This disadvantage is however compensated by the low cost of rice husks, especially in areas where rice is abundant. A simple

engineering system might replace 5-10% of the media daily and might utilize the nutrient rich biosolids for agricultural applications.

The study presented in Chapter 3 demonstrated that EN media, typically used in FBF, is an effective biocarrier in a 3-phase reactor setting. The experiment also showed a VTR of 1219 mg-N/L/day, which is much higher than its design VTR of 750 mg-N/L/day in FBF configurations. The experimental EN reactor displayed a τ of 1.36 g-N/m^3/ppm-day.

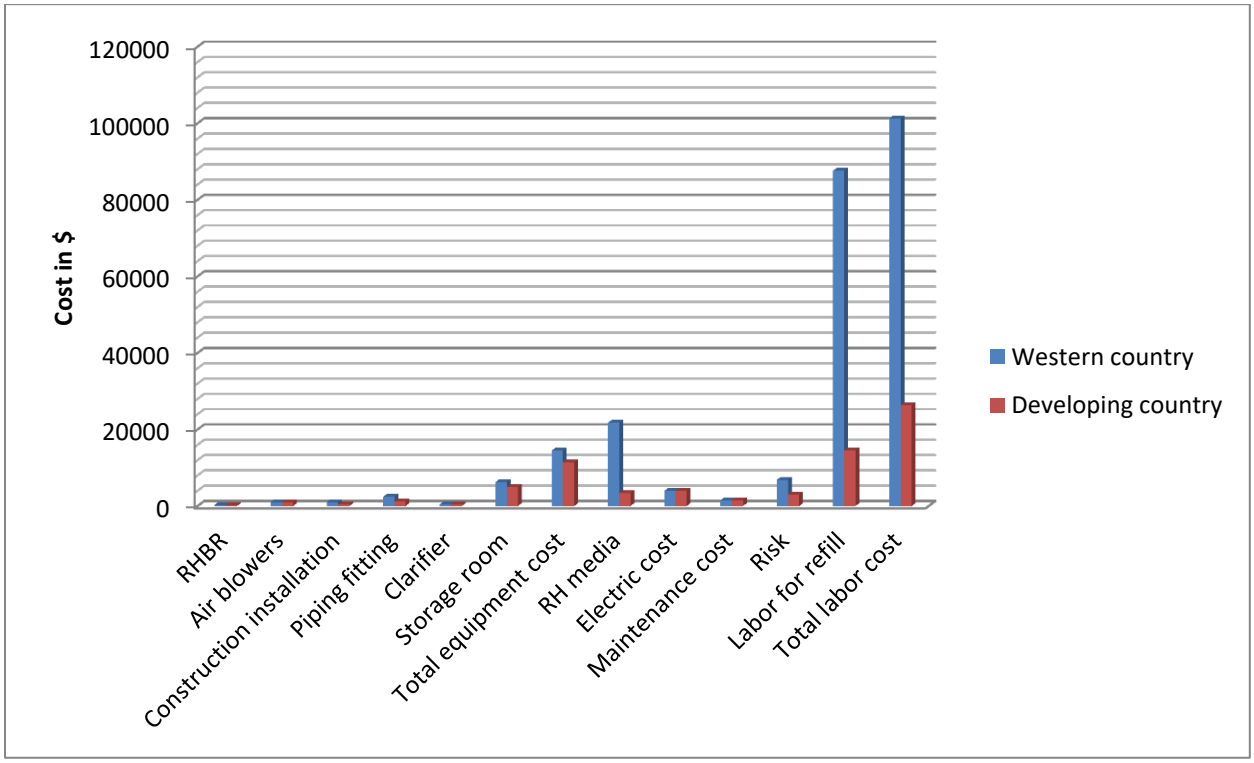

Figure 25: Comparative cost distribution of RHBR-equipped facility ownership costs in the West and in a developing, rice producing country

A cost analysis showed that an EN 3-phase reactor is more profitable than a RHBR in terms of ownership costs in the West, using a U.S., Europe, and Canada economic model. Indeed, a RHBR commercial RAS facility can be expected to be twice as expensive to own than

a facility that uses an EN configuration, mainly because of costs associated with media replacement: additional media, media transport, and storage space. RHBR ownership costs for production and operation. **Error! Reference source not found.** shows that labor and media osts account for the profitability of the system in developing countries primarily.

This study concludes by affirming that RH are a viable biocarrier, and that a RHBR could be a financially valuable asset for third world countries' facilities, in areas with high rice production. This dissertation is part of a larger effort to explore environmentally-friendly aquaculture strategies, and more affordable aquaculture for developing countries that either seek to seize the momentum to expand their already promising production, or have remained marginalized from the fish production market despite strong potential from their other natural resources (i.e., fish, workforce, and rice).

Since the lab experiment showed that the RH were removing TAN consistently at higher loadings, future studies should evaluate RH nitrification capacity for higher trophic conditions (upper mesotrophic and eutrophic), in order to demonstrate the viability of RH biocarriers for growout aquaculture production. We also recommend the building and testing of a RHBR prototype, in order to refine design. This work expolored novel use of RH in a 3-phase reactor which may have significant impact on the development of aquaculture, especially in developing countries.

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APPENDIX A: DESIGN VARIABLES AND EQUATIONS

A.1. Nomenclature

- M_L Mass loading inorganic and organic
- $\mathbf{V}_o$ Overflow velocity for RH
- $\mathbf{V}_b$ Bead volume in L
- $\mathbf{V}_{RH}$ RH volume in L
- $\mathbf{P}_{adj}$ The protein factor (protein ratio)
- F The weight of feed per day in g
- f Fraction of body weight
- E Nitrogen excretion rate
- $\mathbf{Q}_x$ Turnover rate in L/day (required flow)
- $\mathbf{Q}_{tp}$ Throughput flow rate in L/min
- VTR Volumetric TAN rate in mg/day
- HRT_T Tank Hydraulic retention time in min.
- HRT_R Reactor Hydraulic retention time in min.
- $\mathbf{A}_C$ Clarifier cross-section area
- SOR Surface overflow rate for the clarifier in $m^3/m^2/day$
- $\mathbf{V}_{SRH}$ Sinking velocity of RH
- $\mathbf{V}_{FB}$ Floating velocity of beads
- $\mathbf{V}_R$ Reactor volume in L

A.2. Equations utilized for reactor designs

- $\mathbf{L} = \mathbf{F} \times \mathbf{E} \times \mathbf{P}_{adj}$

- $\mathbf{E} = \frac{30\ \text{g–N}}{\text{kg–day}}$
- $\mathbf{F} = \frac{0.4\ \text{kg}}{tank} \times \frac{0.04\ \text{kg feed–day}}{\text{kg–fish}} = \frac{0.016\ \text{kg feed}}{tank–day}$
- $\mathbf{L} = \frac{16\ \text{g feed}}{\text{day}} \times \frac{30\ \text{g–N}}{\text{kg–day}} \times \frac{1\text{kg}}{1000\ \text{g}} \text{x} \frac{50\%}{35\%} \text{x} \frac{1000\ \text{mg}}{1\ \text{g}} = \frac{617\ \text{mg–N}}{\text{day}}$
- $\mathbf{V}_b = \frac{\text{L}}{\text{VTR}}$
- $\mathbf{Q}_x = \frac{\text{L}}{\mathbf{A}_t} = \frac{617\ \text{mg–N}}{\text{day}} \times \frac{1}{0.5\ \text{mg/L}} = \frac{1234\ \text{L}}{\text{day}} \times \frac{1\ \text{day}}{1440\ min} = \frac{0.857\ \text{L}}{\text{day}}$
- $\text{H}_{rt} = \frac{\mathbf{V}_R}{Q_{tp}}$
- $\text{H}_{rt} = \frac{10\ \text{gal}}{\text{tank}} \times \frac{3.785\ \text{L}}{\text{gal}} \text{x} \frac{1}{120\ \text{min}} = \frac{0.32\ \text{L}}{\text{min}}$
- $\text{TAN} = \frac{\text{L}}{\mathbf{2Q}_{pt}} = \frac{617\ \text{mg–N}}{\text{day}} \times \frac{1}{2\ \text{x}\ 0.32\ \text{lpm}} \text{x} \frac{\text{day}}{1440} = \frac{0.669\ \text{mg–N}}{\text{L}}$
- $\mathbf{V}_b = \frac{617\ \text{mg–N}}{\text{day}} \times \frac{\text{L–day}}{750} = 0.823\ \text{L of beads}$
- $\mathbf{V}_o = \frac{\mathbf{Q}_{tp}}{\mathbf{A}_c}$
- $\mathbf{V}_o = \frac{0.32\ \text{Lpm}}{12.75\ in^2} \times \frac{144\ in^2}{ft^2} \text{x}\ \frac{\text{gal}}{3.785\ \text{L}} = \frac{0.955\ \text{gpm}}{ft^2} \times \frac{1440\ \text{min}}{\text{day}} = \frac{1375\ \text{gal}}{ft^2/day}$
- $\mathbf{V}_{SRH} = \frac{0.08\ in^2}{\text{sec}} \times \frac{60\ \text{sec}}{min} \times \frac{1440\ \text{min}}{\text{day}} \times \frac{in^3}{in^2} \times \frac{ft^3}{1728\ in^3} \times \frac{7.48\ \text{gal}}{ft^3} \times \frac{144\ in^3}{ft^2} = \frac{4308\ \text{gal}}{ft^2/day}$

For the bead filter:

- $\mathbf{V}_{FB} = \frac{0.1\ in^2}{\text{sec}} \times \frac{60\ \text{sec}}{min} \times \frac{1440\ \text{min}}{\text{day}} \times \frac{in^3}{in^2} \times \frac{ft^3}{1728\ in^3} \times \frac{7.48\ \text{gal}}{ft^3} \times \frac{144\ in^3}{ft^2} = \frac{5385.6\ \text{gal}}{ft^2/day}$

APPENDIX B: LAB REACTORS' DESIGNS

The following figures present the specific dimensions of both lab reactors.

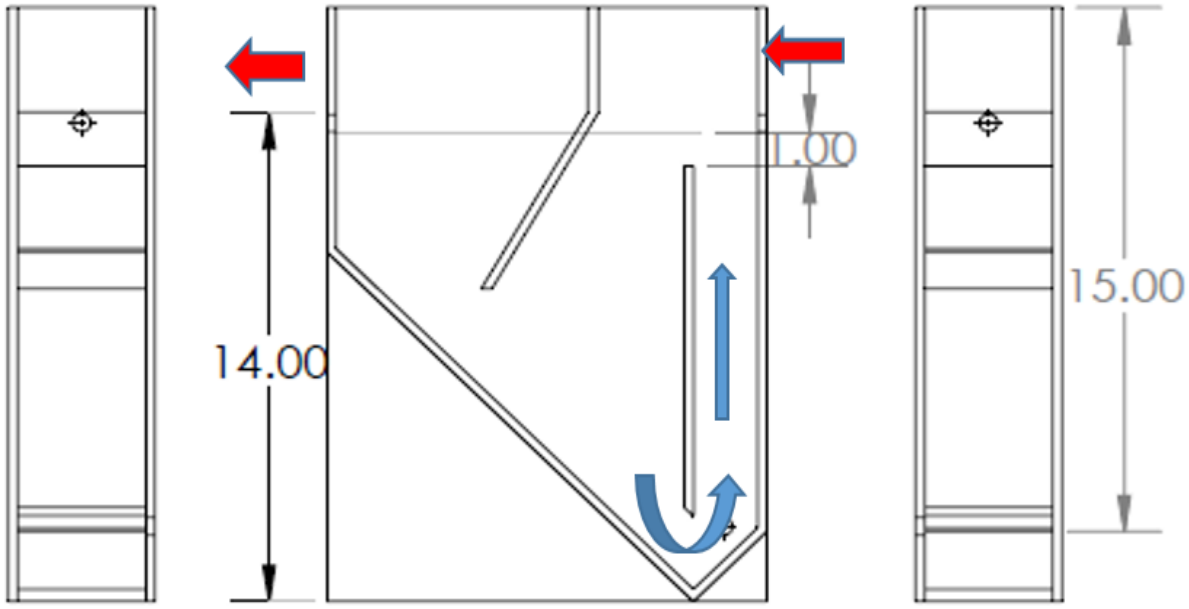

Figure B.1: RH reactors are 16 in. high, 12 in. wide, and 4 in. thick.

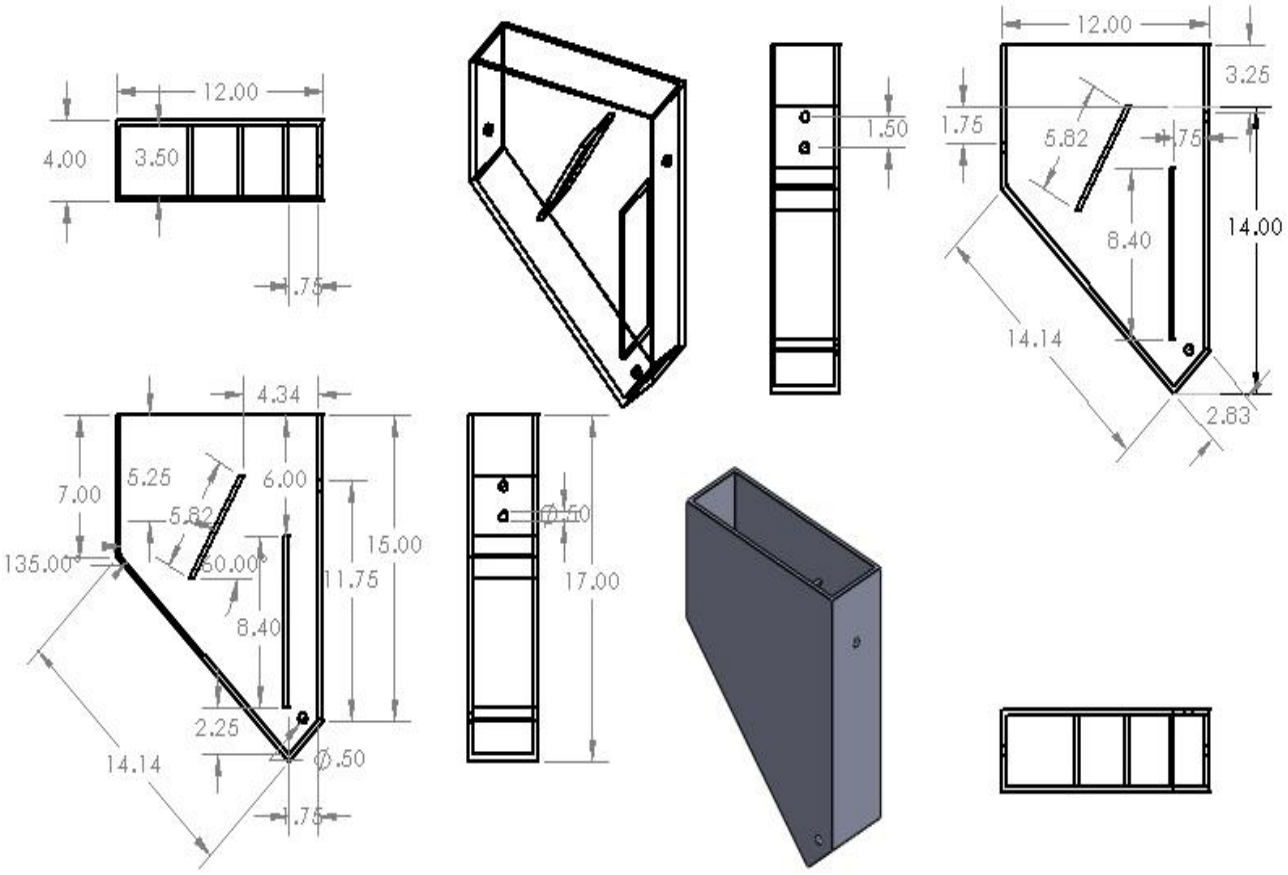

Figure B.2: Interior dimensions of the RH bioreactor amount to a 1 gal. volume.

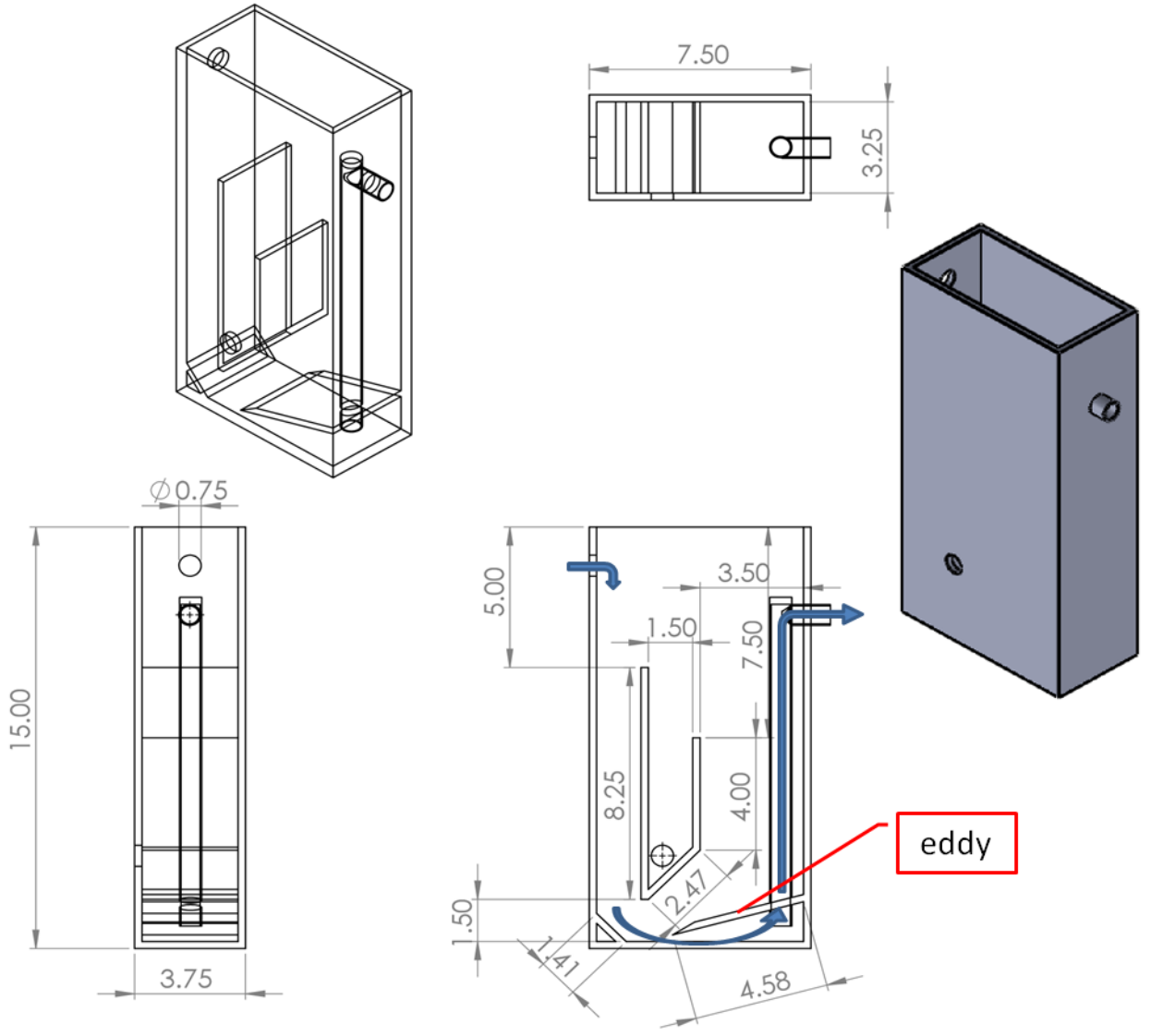

Figure B.3: The eddy at the bottom of the EN 3-P serves as a shield as it prevents the beads from exiting the reactor

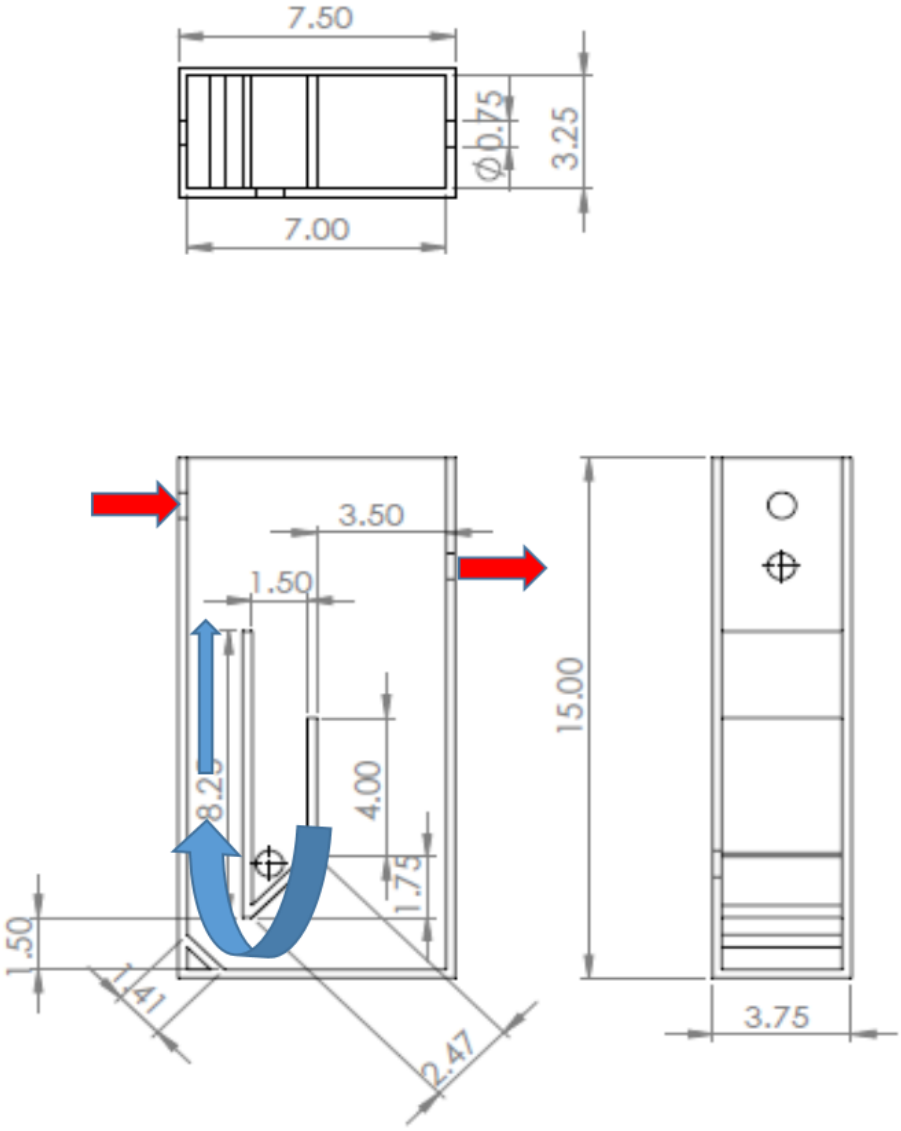

Figure B.4: EN reactors are 15 in. high, 7.5 in. wide, and 3.25 in. thick.

APPENDIX C: MEDIA SINKING AND FLOTATION VELOCITY

The EN floating time is on average 81% the sinking rate of RH.

Table C.1: Media sinking time, floating time, and velocity in seconds

RH sinking (sec)	**RH velocity (ft./sec)**	**EN floating (sec)**	**EN velocity (ft./sec)**
14.091	0.071	9.191	0.109
12.068	0.083	11.145	0.090
11.8	0.085	10.059	0.099
10.333	0.097	7.523	0.133
12.071	0.083	10.461	0.096
13.85	0.072	12.972	0.077
9.724	0.103	13.552	0.074
12.625	0.079	8.52	0.117
9.822	0.102	8.626	0.116
12.006	0.083	8.69	0.115
10.204	0.098	6.792	0.147
14.632	0.068	15.426	0.065
12.331	0.081	9.266	0.108
14	0.071	8.658	0.116
10.225	0.098	10.227	0.098
11.35	0.088	7.98	0.125
12.2	0.082	8.888	0.113
11.225	0.089	7.587	0.132
13.815	0.072	12.548	0.080
11.857	0.084	11.093	0.090
12.0115	**0.085**	**9.9602**	**0.105**

APPENDIX D: ALTERNATIVE COMMERCIAL EN 3-PHASE REACTOR

For comparative purposes, an alternative 3-phase reactor was designed, using a conservative 700 g-N/m^3 VTR. The model is presented in Figure . Corresponding ownership costs were calculated for western aquaculture (Table) and for developing countries, based on the India/Côte d'Ivoire combination economic model (Table).

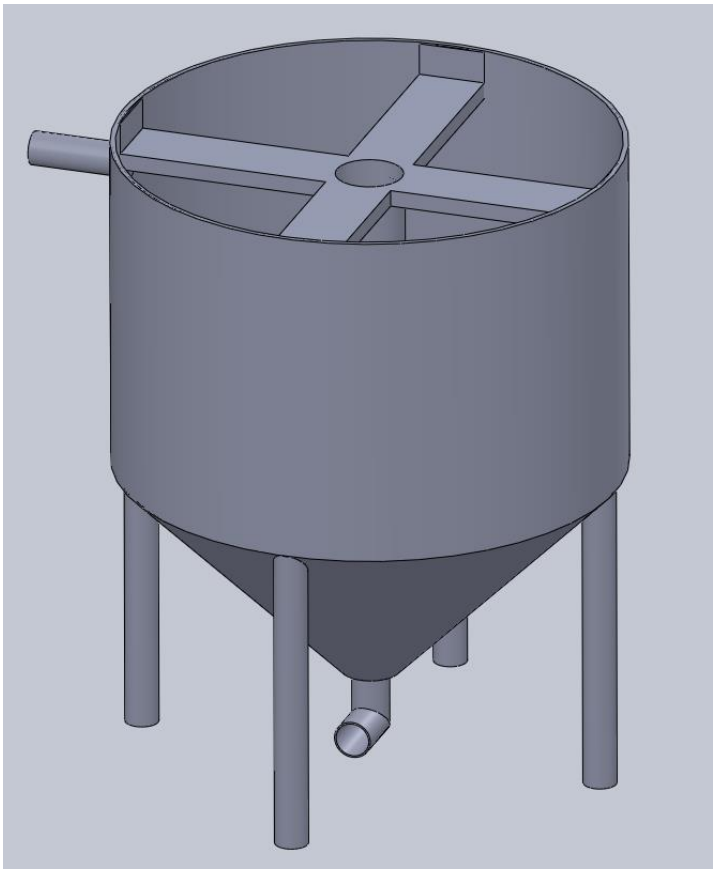

Figure D.1: A 3-phase reactor using EN media eliminates the need for a separate clarifier.

Table D.1: Cost analysis for a RAS facility using an EN3-P reactor in a western country

Annualized Ownership Costs for an EN3-P Fingerling Facility in USA					
Parameters	**Sizing**	**Units**	**Unit Cost**	**PW-Facility**	**Useful Life (yr)**
Equipment					
EN reactor tank	43.00	ft^3	$26.18	$1,125.74	20
Piping PVC plus fittings	500	inches	$0.10	$50.00	20
Air stone	150		$5.00	$750.00	10
Air blowers	100	cfm	$10.08	$1,008.00	7
Construction installation	10	hrs	$25.00	$1,000.00	20
EN media cost	43.00	ft^3	$71.25	$3,063.75	20
Piping fitting	30	hrs/tank	$20.00	$2,400.00	20
Total equipment cost				$9,397.49	
Operation and maintenance					
Electric cost	8760	kwh	$0.18	$13,424.24	
Maintenance cost	150	hrs	$10.00	$12,770.40	
				$26,194.64	
Total cost				$35,592.13	
					20
Number of years	20				
Interest rate	10%		8.5136	0.1598	
Investment interest rate	3%		$4,182.08		
Number of fingerlings	116,298.75	lbs			
Annuity cost	$4,180.64			$0.04	
ownership cost per lb. of fingerlings				$0.05	
			Cost per feed	$0.03	

Table D.2: Cost analysis for a RAS facility using an 3-phase EN reactor in a developing country

Annualized Ownership Costs for an EN3-P Fingerling Facility in Developing Countries							
Parameters	**Sizing**	**Units**	**Unit Cost**	**RAS/Tank**	**Facility**		**Useful Life (yr)**
Equipment							
EN reactor tank	50.00	ft^3	$1.34	$67.00	$268.00		20
Piping PVC plus fittings	500	inches	$0.10	$50.00	$200.00		20
Air stone	150		$5.00	$750.00	$3,000.00		20
Air blowers	100	cfm	$2.35	$235.00	$940.00		20
Construction installation	10	hrs	$10.00	$100.00	$400.00		20
EN media cost	200	ft^3	$71.25	$14,250.00	$57,000.00		20
Piping fitting	30	hrs/tank	$10.00	$300.00	$1,200.00		20
Total equipment cost				**$15,752.00**	**$63,008.00**		
Operation and maintenance							
Electric cost	8760	kwh	$0.45	$3,942.00	$3,942.00		20
Maintenance cost	150	hrs	$10.00	$1,500.00	$1,500.00		20
					$5,442.00		
Total cost				**$15,752.00**	**$63,008.00**		
	Ownership cost per lb. of fingerlings					**0.26**	

APPENDIX E: LIFE-CYLCE COST FOR A COMMERCIAL RHBR

Table E.1: Life-cycle cost analysis over a 20 year period for RHBR-equipped RAS fingerling production facility in a developing country

Initial Investment	Periods	Calculation	Effective annual interest rate	
$500.00	20		3%	
Capital Numerator	(1+i)^n*i	0.0480		
Capital Denominator	(1+i)^n-1	1.0222		
Year	Capital Investment	Future Expenditure		
0	$ $7,342.32	$	Initial Investment cost of plus operation & maintenance in period zero	
1		$ 500.00		Assumed Maintenance of facility
2		$500.00		Assumed Maintenance of facility
3		$500.00		Assumed Maintenance of facility
4		$500.00		Assumed Maintenance of facility
5		$500.00		Assumed Maintenance of facility plus the replacement of equipment with 5 yr. expiration
6		$500.00		Assumed Maintenance of facility
7		$500.00		Assumed Maintenance of facility
8		$500.00		Assumed Maintenance of facility
9		$500.00		Assumed Maintenance of facility
10		$500.00		Assumed Maintenance of facility plus the replacement of equipment with 5 & 10 yr. expiration
11		$500.00		Assumed Maintenance of facility
12		$500.00		Assumed Maintenance of facility
13		$500.00		Assumed Maintenance of facility
14		$500.00		Assumed Maintenance of facility
15		$500.00		Assumed Maintenance of facility plus the replacement of equipment with 5 yr. expiration
16		$500.00		Assumed Maintenance of facility
17		$500.00		Assumed Maintenance of facility
18		$500.00		Assumed Maintenance of facility
19		$500.00		Assumed Maintenance of facility
20		$500.00		Assumed Maintenance of facility plus the replacement of equipment with 5 & 10 yr. expiration
21		$500.00		Assumed Maintenance of facility
NPV	$8,692.83			

VITA

Marlon Greensword graduated from Louisiana State University in August 2010 with a Master of Science in Industrial Engineering, then in August 2015 with a Master of Science in Civil and Environmental Engineering.